JOURNAL OF AGRICULTURAL RESEARCH

VOL. 44 WASHINGTON, D. C., JUNE 15, 1932 No. 12

DEVELOPMENT OF CERTAIN STORAGE AND TRANSIT DISEASES OF CARROT[1]

By J. I. LAURITZEN

Senior Physiologist, Division of Sugar Plant Investigations, Bureau of Plant Industry, United States Department of Agriculture[2]

INTRODUCTION

This paper deals in the main with four diseases of carrot (*Daucus carota* L.), namely, Sclerotinia soft rot (*Sclerotinia sclerotiorum* (Lib.) DBy),[3] Rhizopus soft rot (*Rhizopus tritici* Saito and *R. nigricans* Ehrenb.), bacterial soft rot (*Bacillus carotovorus* L. R. Jones), and Botrytis rot (*Botrytis cinerea* Pers.).

No careful survey of the losses occasioned by these diseases in commercial storage has heretofore been reported. As judged by the reports of various workers and storage men, however, the losses are sometimes large, especially in cases of Sclerotinia soft rot and bacterial soft rot. Data are presented herein showing the losses due to a number of diseases under a variety of storage conditions.

Some idea of the losses caused by these diseases, incident to the shipment of topped carrots to market, may be obtained from Table 1. The data shown in this table were obtained from market-inspection certificates issued by the Bureau of Agricultural Economics, United States Department of Agriculture. The inspections upon which these certificates were based were made in response to a request from one of the parties interested—shippers, carriers, or receivers—and as a result of some question as to condition and grade. The certificates represent the total number of cars of topped carrots inspected from 1922 to 1927, inclusive. The distances covered in the shipment of these carrots ranged from a few miles to many hundred.

The 214 cars inspected represent only about 1 per cent of the 22,195 cars shipped during the same period. Although these shipments include both topped and untopped carrots, the percentage of topped carrots inspected is probably very small. The number of cars inspected is, of course, too small to give an adequate notion of the losses in the total shipment of carrots during this period. Since, however, the inspections were made over a period of years, some idea may be obtained of the occurrence and the relative importance of the several diseases.

[1] Received for publication Oct. 14, 1931; issued July, 1932.

[2] Formerly pathologist, Division of Horticultural Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture.

[3] The usual citation for this combination is (Lib.) Mass.; but Miss Edith K. Cash, of the Bureau of Plant Industry, has found by searching the literature that apparently it was first used by De Bary in Vargleicheude Morphologie der Pilze, p. 33, in 1884, 11 years prior to Massee's use of it.

TABLE 1.—*Losses in 214 carloads of carrots, due to four storage diseases, from 1922 to 1927*

Disease	Cars in which infection occurred		Average percentage of decay based on the number of—	
			Cars in which infection occurred	Cars inspected
	Number	*Per cent*		
Rhizopus soft rot	33	15.4	17	2.6
Sclerotinia soft rot	79	36.9	17	6.3
Bacterial soft rot	32	15.0	8	1.2
Botrytis rot	28	13.1	22	2.9
Soft rot [a]	15	7.0	8	.6

[a] The disease listed under this name may have been any one of the first three diseases.

In some of the cars only one of the four diseases was found; in others, two or more. The average percentage of decay based on the 214 cars is suggestive of the total losses in carrot shipments. The average percentage based on the number of cars in which disease occurred gives a better picture of individual losses. The fact that these percentages are averages indicates that the individual losses varied, sometimes reaching 75 to 100 per cent. This unequal distribution of losses is a hopeful sign. The fact that there was no decay in many cars and slight decay in many others suggests that in these instances the factors favorable to decay were either eliminated or under partial control and that, if the same factors had been controlled or removed in these instances in which the losses were heavy, the total as well as the individual losses would have been smaller.

The data (Table 1) show that the greatest losses were caused by Sclerotinia soft rot, that those caused by Rhizopus soft rot and by Botrytis rot were about equal, and that those caused by bacterial soft rot were the smallest.

Many of the carrots had been stored before they were shipped and probably there were some losses due to decay. In preparing carrots for market it is the usual practice to sort out decayed roots. Losses resulting from sorting must therefore be added to those incurred during transit in computing total losses.

The data set forth herein are based on investigations that had as their object: (1) To make a survey of the diseases that normally affect carrots under a variety of storage conditions; (2) to determine the losses due to these diseases under different conditions of storage; (3) to determine the influence of temperature on the growth of some of the pathogenes in culture media; (4) to study the influence of such factors as temperature, wounds, the presence or absence of organic matter, and the method of infection upon the decay of carrots by the four diseases mentioned above; (5) to test the susceptibility of 18 varieties of carrots to these diseases; and (6) to determine the conditions most favorable for the storage of carrots.

The work was done at the Arlington Experiment Farm, Rosslyn, Va., from 1920 to 1929, inclusive.

APPARATUS AND EQUIPMENT

Most of the work was conducted in infection chambers (*9*) [4] or in storage rooms 8 feet wide, 14 feet long, and 11 feet high. The other equipment used is discussed in connection with the experiments in which it was employed.

MATERIALS

PATHOGENES

The pathogenes employed in the experiments were *Rhizopus tritici* isolated from sweetpotato (*Ipomoea batatas* (L.) Poir.), *R. nigricans* isolated from banana (*Musa paradisiaca* L.), *Sclerotinia sclerotiorum* isolated from cabbage (*Brassica oleracea* L.), *Botrytis cinerea* isolated from carrot, and *Bacillus carotovorus* isolated from carrot.

HOSTS

The Danvers Half Long variety of carrot was used in the infection and storage experiments and in the temperature experiments that had as their object the determination of the cardinal temperatures for infection and decay. The following 18 varieties, with the exceptions noted in the text, were employed in the varietal-susceptibility tests and in some of the storage experiments: Blanche à collet vert (hors terre), Blanche lisse demi-longue, Carter Early Market, Carter Long Forcing, Carter Nantes, Carter Red Elephant, Carter Scarlet Perfection, Carter Summer Favorite, Danvers Half Long, Rouge demi-longue de Danvers, Jaune obtuse du Doubs, Rouge à forcer Parisienne, Rouge demi-courte de Guérande, Rouge demi-longue d'Amsterdam, Rouge demi-longue de Chantenay, Rouge demi-longue Nantaise, Rouge demi-longue de Saint James, and Rouge longue de Saint Valery. The carrots were grown at the Arlington Experiment Farm, Rosslyn, Va., and stored, except when otherwise stated, at a temperature fluctuating between 0° and 2° C.

SOURCES OF CONTAMINATION AND INFECTION

Sclerotinia soft rot differs from Rhizopus soft rot, bacterial soft rot, and Botrytis rot in the fact that its occurrence in storage depends upon field contamination and infection. This disease is common on carrots and a large number of other vegetable crops in the field. If the storage house has not been contaminated by the previous storage of vegetables affected with this disease, uncontaminated or uninfected roots from the field will not become infected by *S. sclerotiorum*.

Harvested carrots apparently are always contaminated with forms of Rhizopus, *Bacillus carotovorus*, and *Botrytis cinerea*, for infection by these pathogenes takes place whenever the roots are stored at certain temperatures and humidities. The sources of contamination are probably both the field and the storage house. All three of these organisms readily grow and reproduce on most, if not all, dead vegetable matter and may be expected to occur whenever such vegetable matter is present. *Bacillus carotovorus* frequently, and *B. cinerea* rarely, affect carrots in the field. In such cases contamination is traceable to field infection.

[4] Reference is made by number (italic) to Literature Cited, p. 911.

EXPERIMENTAL DATA

SCLEROTINIA SOFT ROT

THE PATHOGENE

Ramsey (*12*, *13*) has shown that four species of Sclerotinia may cause decay of carrots under experimental conditions, namely, *S. sclerotiorum*, *S. intermedia* Ramsey, *S. minor* Jagger, and *S. ricini* Godfrey. He states that, of a large number of isolations made from vegetable products on the Chicago market during four years, more than 90 per cent yielded *S. sclerotiorum*. He reports having isolated *S. intermedia* from carrot only on two occasions (*12*, *13*). So far as the writer is aware, *S. minor* and *S. ricini* have not been reported as normally causing decay of carrots. *S. sclerotiorum*, therefore, is probably the principal species causing decay of carrots.

METHOD OF INFECTION

Boyle (*1*) asserts in his work on the Scarlet Runner bean (*Phaseolus coccineus* Jacq.) and broadbean (*Vicia faba* L.) that *Sclerotinia sclerotiorum* obtains entrance to the host tissue by mechanical pressure and that it has the ability to penetrate the cuticle as well as the subcuticular layers. He did not, however, test such tissues as those in the roots of carrots. In the present experiments no effort was made to determine the method by which the pathogene gains entrance to the cells of the carrot, but two experiments (Table 2) were conducted to determine whether there is a normal barrier to the entrance of the fungus into the host tissue.

In these experiments glass tubes 5 mm in diameter and 10 mm long were sealed over fresh wounds, old wounds, areas where secondary rootlets emerge from the primary roots, and the uninjured skin. The tubes were then filled, in the first experiment with sweetpotato decoction, and in the second with carrot decoction, and bits of the mycelium of *Sclerotinia sclerotiorum* were introduced into each tube. The fresh wounds were made by cutting off a slice of tissue from the roots with a sharp knife. The old wounds consisted of areas where the skin had been rubbed or scratched off in the process of harvesting and storing. The roots had been in storage a little over four months in each case. When the roots were to be inoculated over the uninjured skin considerable care was exercised to select areas free from injury. After inoculation the roots were stored at 15° C., for 22 days in the first experiment and for 42 days in the second experiment.

TABLE 2.—*Infection of carrots inoculated with Sclerotinia sclerotiorum over fresh wounds, old wounds, rootlets, and the unbroken skin, and held at a temperature of 15° C.*

Inoculation over—	Storage period		Roots used	Roots infected	
	Experiment 1[a]	Experiment 2[b]			
	Days	*Days*	*Number*	*Number*	*Per cent*
Fresh wounds	22	42	12	10	83
Old wounds	22	42	12	10	83
Rootlets	22	42	13	4	31
Unbroken skin	22	42	23	0	0

[a] The inoculum consisted of bits of the mycelium of *S. sclerotiorum* in sweetpotato decoction.
[b] The inoculum consisted of bits of the mycelium of *S. sclerotiorum* in carrot decoction.

The results in Table 2 show that the unbroken skin of the carrot, if not a perfect barrier, is effective in preventing the entrance of this fungus. If the skin of the carrot root were continuous and uniform throughout, it would effectively limit the amount of decay caused by this pathogene. Unfortunately infection may readily occur at the openings of the tissue associated with the origin of the secondary roots and at the wounds that invariably result from handling in connection with the harvesting and storage of the crop.

Wounding and Infection

In an experiment conducted to measure the influence of wounding at different temperatures, the wounded and unwounded roots were inoculated by dipping them in a suspension of mycelium and agar in water. The inoculum was prepared by squeezing carrot-agar cultures of *Sclerotinia sclerotiorum* through cheesecloth into a quantity of water. The wounding was accomplished by scraping off the skin of the roots, which gave a type of wound common in carrots, especially if roughly handled. A quantity of wounded and unwounded carrots were stored after inoculation at nine different temperatures. Except at one temperature (8° C.), the percentage of infection was larger in the wounded than in the unwounded roots. (Table 3.) Taking the results collectively at the temperatures at which infection occurred, there was 12 per cent more infection in the wounded than in the unwounded roots (32 as compared with 20 per cent). These data, together with those in Table 2, show that wounding is a factor of some importance in the infection and decay of carrots by *S. sclerotiorum*. Incidentally, the results at temperatures 11.5° and 12.5° C. (Table 3) indicate that the relative humidity of the storage chamber may also influence the amount of infection. Fresh wounding would have the effect of increasing the humidity at the wounded surface.

Table 3.—*Infection of wounded and unwounded carrots stored in 12-quart baskets and inoculated with Sclerotinia sclerotiorum at various temperatures*

Temperature (° C.)	Relative humidity	Storage period	Wounded roots			Unwounded roots			Roots stored without treatment	
			Total	Infected		Total	Infected		Total	Infected
	Per cent	*Days*	*Number*	*Number*	*Per cent*	*Number*	*Number*	*Per cent*	*Number*	*Per cent*
33	93	20	(a)	(a)	(a)	(a)	(a)	(a)	(a)	(a)
26.5	91	20	107	0	0	84	0	0	80	0
24	93	20	160	18	11	84	2	2.4	84	0
19	95	20	107	28	26	84	15	18	110	0
18	95	20	90	46	51	109	26	24	117	0
12.5	90	40	101	69	68	75	20	27	99	0
11.5	96	40	121	81	67	106	61	58	89	0
8	96	40	109	7	6	88	10	11	103	0
7	96	40	103	6	6	110	0	0	91	0
3.5	92	40	97	0	0	104	0	0	82	0
Total [b]			791	255	32	656	134	20	693	0

[a] All roots stored at 33° C. were decayed by Rhizopus.
[b] Totals obtained from the figures at the temperatures at which infection occurred.

Influence of Temperature on Growth of the Pathogene on Culture Media

The fungus was grown on carrot agar in 200-cc Erlenmeyer flasks. The flasks were inoculated by introducing into each, with a sterile needle, a small piece of agar containing mycelium from a pure culture. About the same amount of inoculum was used in each flask. Ten flasks were incubated at each temperature. As each lot was inoculated the flasks were placed immediately at the various temperatures. The surface area of the fungus colony was used as a measure of the amount of growth.

The highest temperature at which *Sclerotinia sclerotiorum* was observed to grow was 32.5° C. (Table 4.) No growth took place in 3 days at 35°. This maximum temperature for growth corresponds closely with that obtained by Ramsey (*13*) on potato-dextrose agar, where it grew very slowly at 32° and 33°. Maximum growth occurred at 24° after 3 days. (Table 4.) A slight amount of growth occurred at 0.9° in 22 days in 2 flasks out of 10. No observations were made at temperatures below 0.9°. Growth declined rapidly as the temperature rose above or fell below 24°. (Table 4.)

Table 4.—*Influence of temperature on the growth of Sclerotinia sclerotiorum on carrot agar in Erlenmeyer flasks*

Temperature (° C.)	Period of exposure	Colonies measured	Average area of colonies	Temperature (° C.)	Period of exposure	Colonies measured	Average area of colonies
	Days	*Number*	*Mm² a*		*Days*	*Number*	*Mm² a*
35	3	0	0	12	3	0	0
32.5	3	(b)		12	10	9	2,046
30	3	10	1,017	7.5	10	(c)	
26.5	3	10	2,442	5.5	10	0	0
24	3	10	3,064	7.5	22	(d)	
23.5	3	10	2,430	5.5	22	10	535
21.5	3	10	1,697	2	22	(e)	
19	3	10	773	.9	22	(e)	
15.5	3	10	309				

[a] Mm² is the abbreviation for square millimeter recently adopted for U. S. Government printing.
[b] Growth just started in 1 flask.
[c] Growth just started.
[d] Growth covered flasks.
[e] Growth just started in 2 flasks.

Influence of Temperature on Infection and Decay

To obtain a uniform amount of inoculum for all the roots used in a given experiment and at the same time to confine the initiation of infection to a definite area, so that a comparable quantitative measurement may be made of the decay produced at different temperatures, is more difficult with a fungus like *Sclerotinia sclerotiorum*, which does not normally fruit on culture media, than with fungi that sporulate abundantly. Bits of mycelium, no matter how obtained, are bound to vary. The same is true of bits of sclerotia, although a more uniform quantity of inoculum can be obtained by carefully slicing the sclerotia. For comparisons of the effect of temperature on the amount of decay, both mycelium and sclerotia have been found fairly satisfactory if large numbers of roots are used to eliminate the effects of individual variation. Mycelium inoculation yields a larger percentage of infection, but fairly young sclerotia also yield a large percentage.

The results recorded in Table 5 were obtained from an experiment in which bits of sclerotia were used as the inoculum. The roots were thoroughly washed, a small piece of sclerotium (about 0.5 mm thick by 1 mm square) was inserted in the thickest diameter of each, and the roots were stored at the various temperatures in wire baskets 12 inches in diameter and 12 inches deep.

TABLE 5.—*Influence of temperature on infection and decay of carrots inoculated with Sclerotinia sclerotiorum*

[Bits of sclerotia about 0.5 mm thick by 1 mm square were used as inoculum]

Temperature (° C.)	Storage period	Roots used	Roots infected		Average area of lesions	Temperature (° C.)	Storage period	Roots used	Roots infected		Average area of lesions
	Days	*Number*	*Number*	*Per cent*	*Mm²*		*Days*	*Number*	*Number*	*Per cent*	*Mm²*
32	6	30	0	0	0	10	6	31	0	0	0
28	6	30	22	73	223	14	11	33	32	97	295
25.5	6	33	30	91	474	12	11	34	28	82	284
23	6	34	28	82	541	10	11	31	20	65	58
20	6	33	30	91	504	5	11	31	0	0	0
19.5	6	33	32	97	463	5	41	31	9	29	283
14	6	33	16	48	35	3.5	41	34	2	6	1.6
12	6	34	8	24	11						

The highest temperature at which infection occurred in six days was 28° C. It is not possible to hold carrots at temperatures above 28° except for a short period because of contamination and infection by other fungi. Ramsey (*13*) obtained negative results when young carrot roots were inoculated with *Sclerotinia sclerotiorum* and exposed to a temperature fluctuating between 31° and 33° and averaging 32°. The maximum temperature for infection would therefore seem to be slightly lower than that for growth on culture media. Maximum decay was obtained at a temperature of 23° (Table 5), which corresponds closely to the optimum temperature (24°) for growth on carrot agar. (Table 4.) The discrepancy of 1° may be accounted for by the different temperatures employed in the two cases. The rate of decay declined rapidly as the temperature rose above or fell below 23°.

The lower temperature limit for infection has not been accurately determined, but it is sufficiently near the lower temperature limit for the storage of carrots to make its elimination by the manipulation of temperature impracticable. Ramsey (*13*) has found that *Sclerotinia sclerotiorum* will infect bean pods at a temperature of 0° C. Infection of carrots with this fungus has been obtained at temperatures ranging from 0° to 1°. (Table 7.) Carrots can not be stored at temperatures much below 0° without danger of freezing, the freezing point being about −1.4° (*15*).

If carrots contaminated with this pathogene are stored, it is possible to check the losses by holding the temperature near 0° C., but it is not possible entirely to eliminate decay. The control of this disease should begin in the field.

Relation of Humidity to Infection [5]

Ramsey (*13*) states that moisture is an important factor in the infection of vegetables by *Sclerotinia sclerotiorum*. However, he presents no data on the influence of air humidity on infection.

In an experiment (Table 6) conducted for the purpose of studying the influence of humidity on infection, carrots (Danvers Half Long) grown in the vicinity of Canton, Pa., were used. The roots were obtained directly from the field, stored a few days in a cool basement, and incorporated in the experiment November 30, 1925. Only sound roots and roots relatively free from wounds were used. The roots were stored in chambers 7 by 9 by 10 feet high, provided with ventilation (*14*), and all four chambers were maintained at a temperature of 6.5° C. The relative humidities of these rooms were 95, 90, 80, and 70 per cent, respectively. One 16-quart hamper of untreated roots was placed in each room as checks. Six hampers of carrots were inoculated with *Sclerotinia sclerotiorum* by dipping them in a suspension of mycelium and carrot agar in water. The suspension was made by squeezing six 200-cc Erlenmeyer flasks of carrot-agar cultures (40 cc to the flask) through fine-mesh cheesecloth into 10 gallons of water. After inoculation the roots from four of the hampers were spread out carefully on the floor of a large room and dried with an electric fan. As soon as dry, one hamper of roots was placed in each of the four storage chambers. The two remaining hampers were placed without drying at relative humidities of 90 and 80 per cent, respectively.

In the checks at 95 per cent relative humidity two roots became infected, indicating that there was some contamination of field origin.

Table 6.—*Influence of humidity on infection of carrots inoculated with Sclerotinia sclerotiorum and stored at 6.5° C.*

Relative humidity (per cent)	Storage period	Inoculated roots						Untreated roots		
		Stored without drying			Stored after drying			Total	Infected	
		Total	Infected		Total	Infected				
	Days	*Number*	*Number*	*Per cent*	*Number*	*Number*	*Per cent*	*Number*	*Number*	*Per cent*
95	47				41	4	10	33	2	6
90	47	37	7	19	44	1	2	37	0	0
80	47	42	3	7	39	0	0	38	0	0
70	47				42	0	0	40	0	0
95	104				41	12	29	33	2	6
90	104	37	24	65	44	2	5	37	0	0
80	104	42	10	24	39	0	0	38	0	0
70	104				42	0	0	40	0	0

Infection resulting from inoculation was much more marked in the roots stored in the wet condition than in the roots stored after they were dried. Not only was the percentage of infection higher in the former, but infection developed at a relatively lower percentage of humidity (80 per cent). This increased infection may have been

[5] The results recorded in Tables 6, 27, and 35 were obtained from an experiment conducted at the Marble Laboratory (Inc.), Canton, Pa., through the courtesy of S. M. Marble, who was responsible for maintaining the temperature, humidity, and ventilation throughout the experiment. The results discussed regarding the effect of humidity on infection of carrots by *Bacillus carotovorus* also were obtained in connection with this experiment.

influenced by two factors: (1) The greater amount of water present on the undried roots at the outset of the experiment and (2) the loss of some of the inoculum from the dried roots, due to the drying process and the handling incident to it, although the roots were handled carefully to avoid this. That this pathogene is sensitive to the amount of moisture present is shown by the reduced percentage of infection in the wet roots at a relative humidity of 80 per cent as compared with that at 90 per cent, as well as by the similar effect of the relative humidity on infection in the dried roots.

Varietal Susceptibility to Infection and Decay

To measure the relative susceptibility of 14 varieties of carrots to infection by *Sclerotinia sclerotiorum*, three types of inoculum, two methods of inoculation, and three criteria of measurements were employed.

The three types of inoculum consisted, respectively, of sclerotia, mycelium, and carrots decaying with *Sclerotinia sclerotiorum*. The method of inoculation with either sclerotia or mycelium was to insert, by means of a small scalpel, bits of the inoculum as nearly the same size as possible one-fourth to one-eighth of an inch deep in the thickest diameter of the roots. The method of inoculation with the third type of inoculum was to place near the center of a quantity of carrots one decaying with the pathogene. The percentage of roots infected and the diameter of the lesions were used as measures of susceptibility in the experiments in which the roots were inoculated with bits of mycelium or sclerotium; the percentage of infection and the diameter of the nests that developed were the criteria of susceptibility when the carrots were inoculated with a decaying carrot. There was one replication of each experiment involving each type of inoculum.

The conclusions drawn from all the data obtained are: (1) That all the varieties tested are readily susceptible to decay by *Sclerotinia sclerotiorum*; (2) that, although there is some variation in the number of infections, the percentage of infection, and the degree of decay with the different varieties in a given experiment, these variations are not always paralleled in another experiment conducted as nearly as possible under the same conditions; and (3) that the amount of variation is probably not always a measure of relative susceptibility but is due rather to uncontrollable factors, such as kind and quantity of inoculum, size and shape of roots, variation in the viability of the inoculum, variation in susceptibility within a host variety, degree of wounding, and probably some unknown factors.

The results recorded in Table 7 are submitted as an example of the relative susceptibility of 12 varieties at three different temperatures. The conclusions drawn from these data apply also to the two varieties Rouge demi-longue de Saint James and Rouge longue de Saint Valery, used in some of the other experiments but not in the present one. In this experiment the roots were inoculated by placing a decaying carrot in the center of a 12-quart wire basket of roots of each variety. All the roots were as nearly the same size as possible, and only sound roots that were fairly free from blemishes were used. Considering that in this experiment only one inoculation is involved in the case of each variety at each temperature and that variation in the size and shape of the roots and irregularity in the pack-

ing must of necessity exist in the different baskets, the results obtained in the number of infections, in the percentage of infection, and in the diameters of the nests are regarded as unusually uniform. Certainly all the varieties are readily susceptible and if there is any difference in their susceptibility it is of no practical consequence. The results of experiments previously described confirm this conclusion.

TABLE 7.—*Infection of 12 varieties of carrots inoculated with Sclerotinia sclerotiorum and stored at temperatures of 10°, 4.5°, and 0° to 1° C. for 92, 92, and 155 days, respectively*

Variety	Infection after—											
	92 days at 10°				92 days at 4.5°				155 days at 0° to 1°			
	Roots used	Roots infected		Extent of infection	Roots used	Roots infected		Diameter of nest	Roots used	Roots infected		Diameter of nest
	No.	*No.*	*P. ct.*		*No.*	*No.*	*P. ct.*	*Mm*	*No.*	*No.*	*P. ct.*	*Mm*
Carter Early Market	34	21	62	Infection throughout basket.	46	15	33	112	39	4	10	40
Carter Long Forcing	65	60	92	....do....	75	16	21	76	66	4	6	40
Carter Nantes					76	19	25	108	63	7	11	50
Carter Red Elephant	52	34	65	Infection throughout basket.	52	12	23	127	65	7	11	50
Carter Scarlet Perfection	44	42	95	....do....	52	19	37	108	43	6	14	55
Carter Summer Favorite	52	52	100	....do....	52	19	37	112	55	2	4	45
Danvers Half Long	61	44	72	....do....	66	18	27	102	71	6	8	35
Rouge à forcer Parisienne	79	75	95	....do....	69	28	41	112	74	3	4	45
Rouge demi-courte de Guérande	56	55	98	....do....	84	24	29	102	71	4	6	50
Rouge demi-longue d'Amsterdam	53	45	85	....do....	68	32	47	140	55	6	11	70
Rouge demi-longue de Chantenay	43	39	91	....do....	48	23	48	133	57	4	7	50
Rouge demi-longue Nantaise	53	48	91	....do....	55	19	35	112	50	4	8	55

RHIZOPUS SOFT ROT

SPECIES OF RHIZOPUS RESPONSIBLE FOR DECAY

Rhizopus tritici [6] and *R. nigricans* are the only species of Rhizopus isolated from carrots by the writer. Other species may cause decay (*3*) under special conditions, but since these two species are the only ones obtained from a large number of isolations, it is believed that they are the chief if not the only species that cause decay.

FACTORS AFFECTING DISTRIBUTION OF RHIZOPUS INFECTION AT DIFFERENT TEMPERATURES

DISTRIBUTION IN UNTREATED ROOTS

The results recorded in Table 8 on the distribution of Rhizopus at different temperatures are representative (except for the qualifications given in the text) of experiments conducted during different times of the year for a number of years.

If sound, untreated carrots, directly from the field or after having been stored at 0° to 2° C. for a time, are stored at different temperatures from 0° to 44°, infection by forms of Rhizopus usually occurs at about 15° to 44°. (Table 8.) The lower limit of this range varies

[6] The specific name *tritici* (*9*) as here used may easily include *tritici* itself, *Rhizopus nodosus* Namys., *R. oryzae* Went and Pr. Geerligs, and *R. delemar* (Boid.) Wehmer and Hanzawa, for no morphological characterizations are available by which these species can be definitely separated.

somewhat, depending on the particular lot of carrots. As a rule the amount of infection is relatively small below 30°. Between 20° and 25°, the number of infections is probably influenced by the infection and decay produced by *Bacillus carotovorus*, which is often heavy. The absence of Rhizopus infection at 24° in experiment 2, Table 8, may easily be accounted for by the *B. carotovorus* infection, which amounted to 94 per cent at the end of 19 days. Infection by Rhizopus at temperatures below 20° is erratic in its occurrence, often not appearing at all during the marketable life of the roots and never in large amounts. In only two instances during nearly 10 years of experience in the storage of carrots has the writer observed infection of uninoculated carrots by Rhizopus at temperatures below 12°. At temperatures above 30°, infection by Rhizopus is heavy and occurs in a very short time.

TABLE 8.—*Influence of temperature on infection of carrots by Rhizopus*

EXPERIMENT 1

Temperature (° C.)	Relative humidity	Duration of storage	Inoculated roots[a]			Untreated roots		
			Total	Infected		Total	Infected	
	Per cent	*Days*	*Number*	*Number*	*Per cent*	*Number*	*Number*	*Per cent*
44		11	54	6	11	66	(b)	
41	92	11	70	(b)	(b)	86	(b)	
35	87	11	74	64	86	78	59	76
32	78	11	62	44	71	60	26	43
24.5	96	22	58	1	2	70	3	4
22.5	93	22	79	0	0	58	1	2
20	97	22	61	1	2	81	0	0
18	96	22	60	1	2	69	0	0
14.5	95	31	69	0	0	84	0	0
11.5	95	31	59	0	0	65	0	0
10	98	31	53	0	0	56	0	0
8	90	31	67	0	0	74	0	0
5	100	31	48	0	0	66	0	0
3	100	31	65	0	0	71	0	0

EXPERIMENT 2 (UNTREATED ROOTS)

Temperature (° C.)	Relative humidity	Duration of storage	Total roots	Roots infected by—			
				R. tritici		R. nigricans	
	Per cent	*Days*	*Number*	*Number*	*Per cent*	*Number*	*Per cent*
36.5	93	12	43	35	81	0	0
31	91	19	48	9	19	0	0
24.5	93	27	48	4	8	0	0
24	95	19	57	0	0	0	0
21.5	95	27	59	5	8	0	0
19	92	27	50	3	6	2	4
15.5	91	51	63	0	0	5	8
12.5	95	51	48	0	0	2	4
8	91	79	47	0	0	0	0
6.5	95	113	45	0	0	0	0
2	94	113	45	0	0	0	0
1	91	113	61	0	0	0	0

[a] The inoculum consisted of a spore suspension of *R. tritici* and *R. nigricans*.
[b] Infection by Penicillium was so great at temperatures of 41° and 44° C. as to make a count of Rhizopus infection uncertain, except in the case given.

EFFECT OF TIME

The results recorded in Table 9 were compiled from data obtained in connection with experiment 2, Table 8, to show the effect of time on infection. The period of time employed was governed not only

by infection by Rhizopus but also by infection by *Bacillus carotovorus* and forms of Penicillium and Fusarium, which tends to complicate the problem and to make the selection of comparable periods impracticable. The results recorded in Tables 8 and 9 show quite clearly that carrots are much more resistant to infection by Rhizopus at temperatures below 30° C. than above it.

TABLE 9.—*Influence of time and temperature on normal infection of carrots by Rhizopus*

Temperature (° C.)	Relative humidity	Roots used	Roots infected by Rhizopus after—							
			4 days	5 days	6 days	8 days	12 days	19 days	27 days	51 days
	Per cent	*Number*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
36.5	93	43	26	44	56	70	81			
31	91	48	2	4	4	4	15	19		
24.5	93	48	0	0	0	0	0	4	8	
21.5	95	59	0	0	0	0	2	3	8	
19	92	50	0	0	0	0	0	2	10	
15.5	91	63	0	0	0	0	0	0	8	8
12.5	95	48	0	0	0	0	0	0	0	4

The separate distribution of *Rhizopus tritici* and *R. nigricans* will be discussed later.

EFFECT OF INOCULATION

If carrots are inoculated by being dipped in a spore suspension of *Rhizopus tritici* and *R. nigricans*, the percentage of infection in a given period is usually greater than that of uninoculated roots. (Table 8, experiment 1, and Table 10.)

TABLE 10.—*Infection at various temperatures of wounded and unwounded carrots by Rhizopus*

ROOTS INOCULATED WITH R. TRITICI

Temperature (° C.)	Storage period	Wounded roots				Unwounded roots			
		Total	Infected	Isolations	Organism isolated	Total	Infected	Isolations	Organism isolated
	Days	*Number*	*Number*	*Number*		*Number*	*Number*	*Number*	
32	20	15	14	12	Rhizopus tritici	15	14	9	R. tritici.
25	20	15	5	5	do	15	1	1	Do.
19	32	15	1	0		15	1	1	Do.
15.5	32	15	0	0		15	0	0	
8	32	15	0	0		15	0	0	
5.5	32	15	0	0		15	0	0	

ROOTS INOCULATED WITH R. NIGRICANS

32	20	15	10	8	Rhizopus tritici	15	5	3	R. tritici.
25	20	15	4	0		15	0	0	
19	32	15	1	0		15	0	0	
15.5	32	15	0	0		15	0	0	
8	32	15	0	0		15	0	0	
5.5	32	15	0	0		15	0	0	

ROOTS INOCULATED WITH R. TRITICI PLUS R. NIGRICANS

32	20	15	11	9	R. tritici	15	15	10	R. tritici.
25	20	15	1	1	do	15	0	0	
19	32	15	1	1	R. nigricans	15	0	0	
8	32	15	0	0		15	0	0	
5.5	32	15	0	0		15	0	0	

TABLE 10.—*Infection at various temperatures of wounded and unwounded carrots by Rhizopus*—Continued

ROOTS NOT INOCULATED

Temperature (°C.)	Storage period	Wounded roots				Unwounded roots			
		Total	Infected	Isolations	Organism isolated	Total	Infected	Isolations	Organism isolated
	Days	*Number*	*Number*	*Number*		*Number*	*Number*	*Number*	
32	20	15	4	4	R. tritici	15	6	6	R. tritici.
25	20	15	0	0		15	0	0	
19	32	15	0	0		15	0	0	
8	32	15	0	0		15	0	0	
5.5	32	15	0	0		15	0	0	

Inoculation by dipping in a spore suspension sometimes seems to lower the temperature limit for infection. In the roots in experiment 1, Table 8, inoculated with *Rhizopus nigricans* and *R. tritici*, and in roots (only the unwounded roots are considered in this comparison) inoculated with *R. tritici* alone (Table 10), infection was obtained at a slightly lower temperature than in the uninoculated roots. On the other hand, in roots inoculated with *R. nigricans* alone and with *R. tritici* plus *R. nigricans* the lowest temperature, 32° C. (Table 10), at which infection occurred was the same as in the uninoculated roots. Infection has even occurred at a lower temperature, 12.5°, in uninoculated roots in one experiment (Table 9) than in inoculated roots in another, 18° (experiment 1, Table 8). Aside from the difference in the history of the two lots, there is some difference in the temperatures and periods of time employed, thus making a direct comparison impracticable. The foregoing results justify the conclusion that natural inoculation, so far as the lower temperature limit for infection is concerned, is not a limiting factor in some lots of carrots stored directly from the field, and emphasize the uncertainty of Rhizopus infection at temperatures below 20°. By employing the extreme conditions involved in the "well" method of inoculation [7] (4), it is possible to alter either the normal limits of infection by *R. tritici* and *R. nigricans* or the limits obtained by inoculation with a spore suspension. The lower temperature limits of infection by both pathogenes may thus be lowered and the upper limit of *R. nigricans* raised, until the temperature limits of infection are almost as wide as the temperature limits of growth. The results obtained by this method of inoculation will be discussed later.

EFFECT OF WOUNDING

The data recorded in Table 10 are from one of three experiments conducted to determine the effects of wounding on the infection of carrots by Rhizopus at different temperatures. A different type of wounding was employed in each experiment: (1) Scraping the skin off the root with a knife, (2) striking the root on the side of a wire basket, and (3) cutting a small slice off each root. The last-named type of wounding was used in the experiment reported. In the unreported experiments the wounded and unwounded roots were inoculated by dipping them in a mixed spore suspension of *Rhizopus tritici*

[7] The well method of inoculation consists in placing a 24 to 48 hour old test-tube culture of the organism on liquid medium in a "well" in a root one-half to 1 inch deep. This is made with a ½-inch cork borer and the opening is plugged with cotton after inoculation.

and *R. nigricans*. In the present experiment three spore suspensions were used: *R. tritici* alone, *R. nigricans* alone, and a mixed suspension of the two pathogenes.

The suspensions were prepared by pouring water on cultures of the pathogenes in Erlenmeyer flasks, shaking the flasks to free the spores, and then pouring the suspension through cheesecloth into a larger volume of water to remove as far as possible the mycelium present. The three spore suspensions were made up separately, so that the dosages are not necessarily comparable in the lots inoculated with the different suspensions; but they are comparable in the wounded and unwounded roots inoculated with a given inoculum. The checks consisted of uninoculated wounded and unwounded roots.

At temperatures of 19°, 25°, and 32° C., the only temperatures at which any infection occurred (Table 10), 52, or 29 per cent, of the 180 wounded roots became infected, as compared with 42, or 23 per cent, of the 180 unwounded roots. The margin of infection in the wounded over the unwounded roots was not large (6 per cent), and in two cases the percentage of infection was greater in the unwounded lots.

The results of the three experiments show a somewhat greater percentage of infection in the wounded than in the unwounded carrots, there being exceptions in particular lots. In some instances infection occurred at lower temperatures in wounded than in unwounded roots.

Temperatures at Which Rhizopus Nigricans and R. Tritici Produce Infection

From the data recorded in Table 8 (experiment 2) and Table 10, it appears that 19° C. is near the dividing line between temperatures at which infection is produced by *Rhizopus tritici* on the one hand and *R. nigricans* on the other. *R. tritici* was obtained in all the isolations made from infected roots stored at temperatures above 19°, while *R. nigricans* was obtained from infected roots stored below 19°. These results were confirmed by another experiment made under the same conditions as experiment 2, Table 8.

The foregoing results indicate that, although the amount of inoculum on stored carrots as well as the presence of wounds may increase or otherwise influence infection by *Rhizopus tritici* and *R. nigricans*, artificial inoculation and wounding are not essential to infection. Carrots are very susceptible to decay at temperatures above 30°, much less susceptible between 20° and 30°, and highly resistant below 20°. Carrots are highly resistant to attack by *R. nigricans* under all circumstances.

Effect of Temperature on Infection and Quantity of Decay

The well method was employed to determine the temperature ranges at which *Rhizopus tritici* and *R. nigricans* are able to infect carrots, and to measure the influence of temperature on the quantity of decay during certain periods of time.

Roots of the Danvers Half Long variety were washed in soapy water, rinsed in tap water, and dried in the laboratory. Some were inoculated by introducing 48-hour-old cultures of *Rhizopus nigricans*, grown in 2.5 c c of carrot decoction in test tubes at room temperature,

into wells made in the thickest diameter of the roots, and others by using cultures of *R. tritici* of the same age and grown under the same conditions as the former species. The wells were plugged with sterile cotton and the roots then exposed to the various temperatures. After decay had advanced sufficiently, the carrots were weighed, the decay removed, and the undecayed portion weighed again. The amount of decay at each temperature was obtained by subtracting the second weight from the first.

A number of experiments were made with each pathogene, the results of which are recorded in Tables 11 and 12.

TABLE 11.—*Influence of temperature on infection and decay of carrots inoculated with Rhizopus tritici*

[20 roots used in all cases]

Temperature (° C.)	Storage period	Roots infected	Decay	Temperature (° C.)	Storage period	Roots infected	Decay
	Days	*Number*	*Grams*		*Days*	*Number*	*Grams*
35.5	2	20	283	18.5	2	20	56
33.5	2	20	388	15	2	20	26
32	2	20	341	11.5	2	20	11
30	2	20	335	9.5	2	20	8
28	2	20	218	8	2	0	0
24.5	2	20	161	8	7	20	51
23	2	20	121	5	7	4	6
20	2	20	80	2.5	7	0	0

TABLE 12.—*Influence of temperature on infection and decay of carrots inoculated with Rhizopus nigricans*

[20 roots used in all cases]

Temperature (° C.)	Storage period	Roots infected	Decay	Temperature (° C.)	Storage period	Roots infected	Decay
	Days	*Number*	*Grams*		*Days*	*Number*	*Grams*
35	2	4	[a]	18.5	2	20	249
33.5	2	20	91	13	2	20	150
31.5	2	20	292	12	2	20	34
28.5	2	20	420	9.5	2	20	22
28	2	20	482	8	2	10	[b]
24.5	2	20	465	5	2	0	0
22.5	2	20	419	5	13	0	0
20	2	20	225	2	13	0	0

[a] Just started. Decay at this temperature was due to *Rhizopus tritici*.
[b] Six just started.

It will be seen that both *Rhizopus tritici* and *R. nigricans* are capable of infecting and decaying carrots over a far wider range of temperatures under the conditions of these experiments than under the conditions of the preceding experiments. The obvious differences in the factors operating in the two sets of experiments are the degree of wounding; the amount of inoculum in a given area; and the presence of a medium, in the form of carrot decoction, in the wells in the latter experiments as compared with its absence in the former. An enzyme[8] also may have been present in the carrot decoction to act on the carrot tissue ahead of infection.

It is believed that the degree of wounding was not a limiting factor in the earlier experiments, for carrots have often been subjected to

[8] Observations have shown that the action of *Rhizopus tritici* and *R. nigricans* is the same on carrots as on sweetpotatoes (*2*). This action consists largely in dissolving the middle lamellae through the agency of pectinase secreted by the pathogenes.

various degrees of wounding, dipped in a spore suspension of either species of Rhizopus, and exposed to temperatures below 20° C., with very little resulting infection.

The presence of a greater quantity of inoculum and culture medium in the wells seems to be the factor operating in the present experiments to overcome the resistance of the roots at temperatures at which they are normally resistant.

By the end of two days the amount of decay caused by *Rhizopus tritici* (Table 11) increased from 0 g at 8° C. to 388 g at 33.5° and decreased progressively as the temperature rose above 33.5°. In a supplementary experiment, the maximum amount of decay was obtained at 35.5° and declined as the temperature rose. Some decay occurred at 42°. At 44° the roots showed evidence of injury and were badly infected with Penicillium. Active decay has been found a number of times at a temperature as low as 8°, and *R. tritici* has been isolated from the decay at this temperature. Below 8°, if decay begins at all, it tends to dry up. The lowest temperature at which decay by *R. tritici* has been observed is 5°. This minimum is slightly higher than that (3.5°) observed in sweetpotatoes (*9*).

After two days the amount of decay produced by *Rhizopus nigricans* (Table 12) increased from 0 g at 5° C. to a maximum of 482 g at 28° and decreased progressively as the temperature rose above 28°. It was not possible to determine the maximum temperature for infection of carrots by *R. nigricans*, because of the invasion of *R. tritici*, which infects carrots readily at a temperature of about 35°. In fact it is quite probable that some of the decay at 31.5° and 33.5° was due to *R. tritici*. A large percentage of the isolations made from carrots inoculated as in this experiment have yielded *R. tritici*. In the present case *R. tritici* alone was obtained at 35°. Isolations were not made from roots stored at the other temperatures. The lowest temperature at which decay by *R. nigricans* has been found is 8°.

Rhizopus Infection in Carrots and Sweetpotatoes

A comparison of infection of carrots and sweetpotatoes (*9*, *10*) by species of Rhizopus brings out some interesting differences and similarities.

(1) Fresh wounds are almost invariably essential to infection of sweetpotatoes at temperatures below 33° C. Some infection will occur without wounding above 33°. Wounds have very little effect on infection of carrots at any temperature. (2) Some change takes place in sweetpotatoes when exposed to temperatures above 33° that makes them fairly susceptible to decay without wounding. Some change takes place in carrots when exposed to temperatures above 30° that makes them very susceptible to decay. (3) *Rhizopus tritici* and *R. nigricans* cause most if not all the decay in both cases. (4) The total temperature range at which Rhizopus has been found to infect carrots (0°–2° to 44°) and that at which it infects sweetpotatoes (3.5° to 44°) are almost identical. The difference in the two cases is probably due to differences in the temperatures employed in the two sets of experiments and possibly to complications introduced by the effects of other fungi on sweetpotatoes at temperatures near 3.5°. (5) *R. tritici* causes practically all the decay of carrots, whereas *R. nigricans* causes most of the decay of sweetpotatoes, largely because sweetpotatoes as a rule are held at temperatures below 20° at which

R. nigricans normally causes all the decay. *R. tritici* causes all the decay of sweetpotatoes above 32°, and the infection is divided between the two species at temperatures between 20° and 32°. (6) The normal range of temperature at which *R. tritici* infects wounded and unwounded carrots extends from about 19° to 44°, and the range at which it infects wounded sweetpotatoes is from 20° to 44°. (7) Wounded sweetpotatoes at temperatures below 26° are very susceptible to infection by *R. nigricans*, whereas wounded or unwounded carrots are highly resistant to infection by this pathogene. (8) The resistance of sweetpotatoes to infection by Rhizopus resides in the skin and healed-over wounded surfaces, and fresh wounding removes this resistance (*11*); whereas the resistance of carrots to infection by Rhizopus is affected very little by wounding. The normal resistance of sweetpotatoes to infection by *R. tritici* at temperatures below 20° is broken down when the roots are inoculated by the well method; the resistance of carrots to *R. tritici* at temperatures below 20° and to *R. nigricans* at temperatures from 8° to 33.5° is broken down when the roots are inoculated by the well method.

Varietal Susceptibility to Decay

susceptibility to rhizopus nigricans

It has already been shown that either the Danvers Half Long variety is normally resistant to infection and decay by *Rhizopus nigricans* or that this pathogene is not well equipped to infect the roots under normal conditions. The following varieties of carrot have been found to be susceptible when inoculated by the well method with 24-hour to 48-hour cultures grown in 2.5 c c of carrot decoction in test tubes, plugged with cotton, and stored at temperatures fluctuating from 10° to 15° C.: Blanche à collet vert (hors terre), Blanche lisse demi-longue, Carter Early Market, Carter Long Forcing, Carter Nantes, Carter Red Elephant, Carter Scarlet Perfection, Carter Summer Favorite, Danvers Half Long, Rouge demi-longue de Danvers, Jaune obtuse du Doubs, Rouge à forcer Parisienne, Rouge demi-courte de Guérande, Rouge demi-longue d'Amsterdam, Rouge demi-longue de Chantenay, Rouge demi-longue Nantaise, and Rouge longue de Saint Valery. The degree of susceptibility does not seem to vary greatly under these conditions.

During one to four different seasons all these varieties were stored at temperatures ranging from 0° to 15.5° C. The only infection recorded, aside from that of Danvers Half Long by Rhizopus, was on Rouge demi-longue de Danvers held at a temperature of 10° during the season of 1926–27, when about 5 per cent of the stock stored (a little more than a peck) was decayed. This variety was stored the two preceding seasons without infection by Rhizopus. This variety is really a strain of Danvers Half Long, and in general there has been found very little difference between the two in their reaction to disease. Although no isolations were made from these roots, because they were completely decayed at the time of the inspection, it is believed, because of earlier experience, that the decay was due to *R. nigricans*. In any case, the varieties listed above probably can safely be stored at temperatures below 15° without much danger of infection by *R. nigricans*.

130648—32——2

SUSCEPTIBILITY TO RHIZOPUS TRITICI

In two experiments conducted to determine the susceptibility of 16 varieties of carrots to infection and decay by *Rhizopus tritici*, the roots were inoculated by dipping them in a spore suspension, after which they were stored at a temperature of 30° C. The percentage of infection in most varieties was larger in the second experiment (Table 13) than in the first in spite of a shorter storage period. This difference was probably due in part to the heavier spore suspension used in the second experiment. All 16 varieties were found to be susceptible. There was considerable variation in the percentage of infection in the different varieties in a given experiment, but the variation was not always parallel to that of the other experiment. Moreover, the degree of variation in a given variety in the two experiments was sometimes as great as the variation in the different varieties in the same experiment. It is believed, therefore, that there is not a marked difference in the susceptibility of the varieties.

TABLE 13.—*Infection in 16 varieties of carrots inoculated by dipping in a spore suspension of Rhizopus tritici and stored at 30° C.*

Variety	Experiment 1					Experiment 2				
	Total roots	Roots infected after—				Total roots	Roots infected after—			
		7 days		15 days			3 days		8 days	
	Number	*Number*	*Per cent*	*Number*	*Per cent*	*Number*	*Number*	*Per cent*	*Number*	*Per cent*
Blanche à collet vert (hors terre)	32	6	19	16	50	21	15	71	16	76
Blanche lisse demi-longue	30	1	3	21	70	22	14	64	19	86
Carter Early Market	70	10	14	34	49	58	10	17	30	52
Carter Nantes	70	9	13	48	69	44	26	59	37	84
Carter Red Elephant	51	8	16	24	47	47	21	45	36	77
Carter Scarlet Perfection	48	2	4	17	35	30	13	43	22	73
Carter Summer Favorite	42	13	31	37	88	23	14	61	20	87
Danvers Half Long	61	7	11	36	59	48	17	35	40	83
Rouge demi-longue de Danvers	56	25	45	46	82	33	13	39	32	97
Jaune obtuse du Doubs	38	4	11	21	55	33	17	52	19	58
Rouge à forcer Parisienne	86	53	62	84	98	29	7	24	22	76
Rouge demi-courte de Guérande	48	13	27	45	94	32	14	44	30	94
Rouge demi-longue d'Amsterdam						41	14	34	38	93
Rouge demi-longue de Chantenay	57	10	18	36	63	33	19	58	27	82
Rouge demi-longue Nantaise	63	11	17	36	57	30	7	23	25	83
Rouge longue de Saint Valery	55	13	24	29	53	40	21	53	26	65

In two other experiments with these same varieties the roots, after being washed in soap and water, rinsed in tap water, and dried in the laboratory, were inoculated by introducing a 48-hour culture of *Rhizopus tritici* into wells. The wells were made in the thickest diameter of the root, penetrated to the center, and were plugged with cotton. Twenty roots of each variety were employed in each experiment. All the varieties were stored in wire baskets under the same conditions in a room 8 feet square and 10 feet high, provided with ventilation at a temperature of about 23° C. After two days in the first experiment and three days in the second, the roots of each variety were weighed separately, the decay removed, and the undecayed portion weighed again. The weight of the decay was obtained by subtracting the second weight from the first.

The percentage of infection in both experiments was 100, except in the case of the variety Rouge demi-longue de Chantenay, in

which one root in one experiment remained uninfected during the time employed.

Although there is some variation in the amount of decay in the different varieties in a given experiment, the variations do not always correspond in the two experiments. Nor is the difference in the amount of decay in the several varieties large enough to indicate marked resistance on the part of any.

The results of the two types of experiments show that all the varieties are very susceptible to infection by *Rhizopus tritici* under the same conditions as those under which Danvers Half Long was found to be readily susceptible, that is, when the roots are inoculated with a spore suspension and stored at a temperature of 30° C. and above, and when they are inoculated by the well method.

BACTERIAL SOFT ROT (SLIMY SOFT ROT)

Bacillus carotovorus is always present to some extent on vegetables in storage and transit. In addition to decaying carrots in storage and transit, it intermittently occasions losses by attacking the roots in the field. Decay in the field seems to depend on rather special conditions, such as high temperature combined with a high water content of the soil following a severe attack of blight (*Macrosporium carotae* Ell. and Langlois). Although its presence in storage and transit as well as the amount of loss it produces may be influenced by its occurrence in the field, it is not absolutely dependent upon such occurrence.

The Pathogene

In the experiments in which the carrot roots were inoculated, the Jones 3A strain of *Bacillus carotovorus* was employed. In the temperature and infection experiments in which no inoculum was used, it is assumed that all the bacterial decay that occurred, except at temperatures of 38° to 40° C. and above, was due to *B. carotovorus*.

Method of Infection

According to Jones (*6*), infection of carrots by *Bacillus carotovorus* does not normally occur through the unbroken skin. Unfortunately the skin is not continuous over the entire surface of the roots. Its continuity is interrupted by the wounds produced by harvesting operations, including injury to root tips and secondary roots. These wounds permit of a certain amount of infection whenever conditions of temperature and moisture are favorable. (Tables 14 and 15.)

TABLE 14.—*Normal infection by Bacillus carotovorus of wounded and unwounded carrots stored at various temperatures for 31 days*

Temperature (° C.)	Wounded roots			Unwounded roots		
	Total	Infected		Total	Infected	
	Number	*Number*	*Per cent*	*Number*	*Number*	*Per cent*
24	64	58	91	75	50	67
22.5	71	18	25	81	18	22
19.5	66	20	30	75	10	13
17.5	73	5	7	76	2	3
15	78	0	0	78	0	0
Total [a]	274	101	37	307	80	26

[a] Taken from results at temperature at which infection occurred.

TABLE 15.—*Influence of temperature and humidity on infection of uninoculated carrots by Bacillus carotovorus*

Experiment 1 [a]						Experiment 2 [b]					
Temperature (° C.)	Relative humidity	Storage period	Roots used	Roots infected		Temperature (° C.)	Relative humidity	Storage period	Roots used	Roots infected	
	Per cent	*Days*	*Number*	*Number*	*Per cent*		*Per cent*	*Days*	*Number*	*Number*	*Per cent*
41	92	3	86	0	0	36.5	93	12	43	3	7
35	57	11	78	3	4	31	91	19	48	12	25
32	78	13	60	1	2	24.5	92	27	48	32	67
24.5	96	22	70	41	59	24	92	19	57	54	95
22.5	93	22	58	14	24	21.5	95	27	59	25	42
20	97	22	81	13	16	19	92	27	50	18	36
18	96	22	69	10	14	15.5	91	51	63	11	17
14.5	95	22	84	0	0	12.5	95	51	48	7	15
11.5	95	22	65	0	0	8	91	79	47	0	0
10	98	31	56	0	0	6.5	95	113	45	0	0
8	90	31	74	0	0	2	94	113	45	0	0
5	100	31	66	0	0	1	91	113	61	0	0
3	100	31	71	0	0						

[a] Roots exposed to various temperatures after being stored at a temperature of 0° to 2° C., from Nov. 1 to Mar. 4.

[b] Newly dug roots exposed to various temperatures.

WOUNDING AND INFECTION

Normally the range of temperature extending from 15° to 25° C., and particularly temperatures above 20°, are favorable to infection of carrot by *Bacillus carotovorus*. A quantity of unwounded roots and roots wounded by being struck three times against the blunt edge of a wire basket were stored without inoculation for 31 days at a number of temperatures from 15° to 24°, inclusive. The wounded roots showed a higher percentage of infection than the unwounded roots at all temperatures at which infection occurred. (Table 14.) These results indicate that, although wounding influences infection, heavy infection may take place in the absence of fresh wounding.

TEMPERATURE RELATIONS

GROWTH OF THE PATHOGENE

Jones (*6*) found the maximum temperature for growth of *Bacillus carotovorus* on culture media to be slightly below 39° C. Very little growth took place in 20 days at temperatures ranging from 0.6° to 1°. Some growth took place at 2° in 24 hours. The optimum temperature for growth was reported to be approximately 27° to 30°.

EXPERIMENTS ON INFECTION AND DECAY

Three types of experiments were employed in the study of the relation of temperature to the infection and development of decay by *Bacillus carotovorus*. In type 1, sound untreated roots were stored at a series of temperatures in infection chambers and storage rooms; in type 2, a root decaying with *B. carotovorus* was placed at or near the center of a quantity of sound roots and the roots were stored at various temperatures; in type 3, the roots were inoculated by the well method and stored at various temperatures. The objects of the three types of experiments were as follows: Type 1, to determine the normal temperature range for infection and decay and the time required for infection to occur; type 2, to study the effects of con-

tamination by decaying roots on infection and decay at various temperatures; and type 3, to measure the influence of temperature on the quantity of decay at different temperatures.

TYPE 1.—The behavior of carrots stored at different temperatures in regard to infection by *Bacillus carotovorus* within any given period of time is not always uniform either in the range of temperatures at which infection occurs or in the amount of decay present. Aside from the effects of temperature and wounding, the extent of decay is probably influenced by the following factors: The particular lot of carrots stored, the amount of contamination, the humidity of the storage room, and decay by other pathogenes.

The difference in behavior of different lots of carrots and the effects of decay by other pathogenes on decay by *Bacillus carotovorus* will become obvious as the discussion continues. It is a matter of general observation that roots contaminated because of field infection are more likely to decay than roots from uncontaminated fields. A high humidity in the storage room seems to favor infection, although there are no data available to show its exact relation to infection.

The results given in Tables 15 and 16 are submitted as examples of what may happen in regard to infection by *Bacillus carotovorus* if roots are stored at a range of temperatures from 0°–2° to 41° C. In experiment 1 (Table 15) the roots were placed at the various temperatures after having been stored at a temperature of 0° to 2° from November 1 to March 4, while those in experiment 2 were stored at the various temperatures at harvest time. Aside from the difference in the age of the roots at the time of storage, the conditions of the two experiments are not entirely parallel. There are differences in temperature, humidity, and time. The differences in temperature are not believed to be of any importance. The low humidities at the temperatures of 32° and 35° in experiment 1 of Table 15 may possibly operate to reduce the percentage of infection. The temperatures and relative humidities between 15° and 25° employed in the two experiments are sufficiently alike to be fairly comparable. At these temperatures there is a difference in time of three days between experiment 1 (Table 15) and the 19-day period in Table 16. At these temperatures the percentage of infection in newly dug carrots in 19 days (Table 16) was in most instances about the same as in the older roots in 22 days, and at 24° the newly dug carrots showed a much larger percentage of infection than was shown by the older roots at the nearest corresponding temperature. (Table 15, experiment 1.) These data indicate at least that the roots do not necessarily increase in susceptibility with age. The lower limits for infection in the periods of time considered were about the same. The percentage of infection over the entire infection range in the two experiments was undoubtedly influenced by infection by Rhizopus and Fusarium, especially the former. Infection by any one of these organisms always shows some variation in amount. Consequently any variation in the percentage of infection by one would tend to cause a variation in the others.

TABLE 16.—*Influence of time on infection of uninoculated carrots by Bacillus carotovorus at various temperatures*

Temperature (° C.)	Relative humidity	Roots used	Roots infected after—							
			4 days	5 days	6 days	8 days	12 days	19 days	27 days	51 days
	Per cent	*Number*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
36.5	93	43	0	2	5	7	7	----	----	----
31	91	48	0	6	6	6	21	25	----	----
24.5	93	48	0	2	2	2	17	54	67	----
24	95	57	----	----	----	----	54	95	----	----
21.5	95	59	0	2	2	2	8	15	42	----
19	92	50	0	0	0	0	6	14	36	----
15.5	91	63	0	0	0	0	0	0	5	17
12.5	95	48	0	0	0	0	0	0	0	15

Uninoculated carrots have been infected by *Bacillus carotovorus* at temperatures from 0°–2° to 36.5° C. (Table 15, experiment 2, and Table 17.)[9] In only one year has bacterial soft rot been observed at a temperature of 0° to 2° during nine years of storage of the Danvers Half Long variety taken directly from the field. After 131 days of storage the percentage of infection was 0.2 in this variety but reached as high as 8 in one other variety.

TABLE 17.—*Normal infection by Bacillus carotovorus of the Danvers Half Long variety of carrots stored at several temperatures and humidities during four seasons*

Storage season	Temperature	Relative humidity	Storage period	Roots used	Roots infected	
	°C.	*Per cent*	*Days*	*Number*	*Number*	*Per cent*
1924–25	10	80–96	102	1,288	80	6
	7	80–96	102	1,190	9	0.8
	4.5	75–83	102	1,029	0	0
	0–2	91	102	1,412	0	0
1925–26	10	75–85	121	437	58	13
	7	75–85	121	411	3	0.7
	0–2	90	121	886	0	0
1926–27	15.5	90	39	66	22	33
	10	80	99	58	11	19
	4.5	70	165	52	0	0
	0–2	90	131	192	0	0
	0–2	90	131	978	2	0.2
1927–28	12.5	91	50	121	53	44
	12.5	91	70	267	205	77
	4.5	84	50	134	0	0
	4.5	84	100	133	5	4
	4.5	84	150	124	0	0
	0–2	90	50	135	0	0
	0–2	90	100	129	0	0
	0–2	90	150	136	0	0
	0–2	86	50	131	0	0
	0–2	86	100	125	0	0
	0–2	86	150	134	0	0

Likewise, infection at 4.5° C. was limited to one season out of three seasons' storage of the Danvers Half Long variety (Table 17), the percentage of infection being 4 after 100 days of storage. In another season 1 per cent of each of four other varieties was infected after 92 days of storage at 4.5°. Infection at temperatures below 10° has always been relatively small even after long periods of storage. At a temperature of 10° and above, the percentage of infection may

[9] The results recorded in Table 17 were obtained from the storage of carrots at temperatures from 0°–2° o 15.5° during four seasons. The roots were taken directly from the field and stored either in 16-quart ampers or bushel crates in storage rooms 8 by 14 by 11 feet high.

become high if the duration of the storage period is long enough. With the rise in temperature from 8° to about 21.5°, there is a shortening of the time required for infection to occur. (Table 15, experiment 2, and Table 16.) At temperatures between 21.5° and 36.5° no difference is apparent in the time required for infection to take place, unless a slightly higher percentage of infection at 31° (Table 16) indicates slightly earlier infection. There is very little divergence in the number of infections occurring at these temperatures during the first 8 days. The optimum temperatures for infection among those used (Table 15) were 24° and 24.5°. It is to be expected that the optimum temperature for infection will vary if different temperatures are employed.

TYPE 2.—When bacterial soft rot is present in the field, decaying roots may sometimes be stored with healthy stock and shipped with them to terminal markets, where the usual storage temperatures range from 0° to 2° C. The results in Table 18 were obtained from an experiment designed to throw some light on such cases, as well as on what would happen if such contaminated stock were stored at different temperatures.

In this experiment, eleven 12-quart baskets of sound carrots were inoculated by placing a decaying root in the center of each. The baskets were then stored at temperatures ranging from 2° to 31.5° C. and at relative humidities ranging from 80 to 98 per cent. The rot was the result of inoculation with a pure culture of the Jones 3A strain of *Bacillus carotovorus*. This experiment has some limitations in so far as measuring the influence of temperature on the amount of decay is concerned. Among these are variation in the length of the storage period; variation in the size, shape, and packing of the roots about the inoculum; and the fact that there was only one inoculation at each temperature.

It was desired to obtain infection over the widest possible range of temperature. In attempting to extend infection to as low a temperature as possible, complications developed at the three highest temperatures where the roots showed considerable decay from Rhizopus by the end of the twenty-second day.

TABLE 18.—*Influence of temperature on infection of carrots inoculated by placing a root decaying with Bacillus carotovorus in the center of each hamper*

Temperature (° C.)	Relative humidity	Storage period	Roots used	Roots infected		Temperature (° C.)	Relative humidity	Storage period	Roots used	Roots infected	
	Per cent	*Days*	*Number*	*Number*	*Per cent*		*Per cent*	*Days*	*Number*	*Number*	*Per cent*
31.5	90	22	31	2	6	15.5	95	29	57	11	19
30	86	22	36	4	11	12	90	29	70	7	10
24.5	88	22	45	9	20	8	93	29	49	0	0
23.5	91	29	59	12	20	8	93	80	49	7	14
21	96	29	36	21	58	5.5	98	80	46	3	7
19	80	29	67	15	22	2	95	80	45	1	2

Obviously, the normal variation in size and shape of the carrots and in the packing of them in the baskets would give rise to some variation in the number of roots in a given area about the inoculum. There is always some variation in the amount of decay resulting from a single inoculation. Although the effect of the difference in tempera-

ture tends to overshadow this variation, it does not always do so, and some effects of the initial variation always persist (*8*). Numbers alone will compensate for this variation.

Infection (Table 18) occurred over the entire temperature range. At 8° C. there was no infection in 29 days, but in 80 days there was 14 per cent. The maximum number of infections took place at 21°. This temperature is 3.5° below the optimum obtained in experiments 1 and 2, Table 16. It is believed that 24.5° more nearly represents the optimum for infection and decay. This opinion is confirmed by the results discussed under experiments of type 3, where more quantitative methods were employed. Infection at 8°, 5.5°, and 2° occurred rather slowly. Only 7 roots at 8°, 3 at 5.5°, and 1 at 2° became infected in 80 days. However, in contrast to the results obtained from uninoculated roots, infection occurred at these temperatures in a shorter period of time, in larger amounts, and apparently with greater certainty. At some of the higher temperatures the infection in the same period of time was heavier in uninoculated roots (Table 16) than in the inoculated roots of the present experiment. It is not clear why this should be so, unless the normal contamination aside from the root used for inoculation was less in the latter case than in the case of the uninoculated roots. (Compare results in Table 16 with those in Table 18.) These results show that the inclusion of decaying carrots in stored lots may be a source of decay, the amount depending on the quantity of decaying material present, the temperature of the storage room, and the length of the storage period. This method of inoculation insures infection over a wider temperature range, particularly at low temperatures.

TYPE 3.—For this experiment 180 sound roots of the Danvers Half Long variety were selected for uniformity of shape and size. The results of the test are recorded in Table 19. The roots were first thoroughly washed in soapy water and dried. One hundred and twenty were then inoculated by introducing 0.5 cc of a suspension of *Bacillus carotovorus* in beef bouillon into a well, 8 mm in diameter, penetrating to the center of each root. The wells were plugged with cotton and 10 roots were stored at each of the 12 temperatures shown in Table 19. In 60 roots used as checks sterile beef bouillon, instead of the bacterial suspension, was placed in the wells. Five of these roots were placed at each of the 12 temperatures. The amount of decay was determined by weighing the decaying roots, removing the decay, weighing the undecayed portion, and subtracting the second weight from the first. There was no infection in any of the checks. The highest temperature at which infection occurred in three days was 29° C. This temperature is regarded as the maximum at which decay will take place only for the time limit and other conditions of this experiment. Infection has been obtained at 36.5° (Table 15, experiment 2), but no infection occurred at 41° (Table 15, experiment 1). Thirty-five degrees is 4° below the maximum temperature obtained by Jones (*6*) for growth of *B. carotovorus* on culture media. The maximum temperature for the infection of carrots by *B. carotovorus* is difficult to determine because of infection by Rhizopus, which is always heavy at temperatures above 30°, even within the limits of a few days.

TABLE 19.—*Influence of temperature on infection and decay of carrots inoculated by the well method with Bacillus carotovorus*

[10 roots used in all cases]

Temperature (° C.)	Storage period	Roots infected		Decay	Temperature (° C.)	Storage period	Roots infected		Decay
	Days	*Number*	*Per cent*	*Grams*		*Days*	*Number*	*Per cent*	*Grams*
35	3	0	0	0	15.5	3	9	90	21
32	3	0	0	0	12	3	5	50	5
29	3	2	20	9	8	3	0	0	0
26.5	3	6	60	42	8	7	5	50	4
24.5	3	8	80	50	5.5	7	1	10	* 0
21.5	3	10	100	41	2	12	0	0	0
18.5	3	10	100	34					

* Decay not measurable.

The greatest amount of decay occurred at 24.5° C. (Table 19; see also Table 15.) The amount of decay declined as the temperature rose above or fell below 24.5°. This optimum is slightly below that (27° to 30°) for growth on culture media. The lowest temperature at which infection was obtained in this experiment was 5.5° Time was undoubtedly a limiting factor.

The temperature at which infection of carrots by *Bacillus carotovorus* occurred in the three types of experiments ranged from 0°–2° to 36.5° C. The optimum temperature for infection is about 25°.

Influence of Relative Humidity on Infection and Decay

Only one experiment was conducted to determine the relation of humidity to infection of carrots by *Bacillus carotovorus*. Unfortunately the temperature used (6.5° C.) is normally a limiting factor in infection by this pathogene, and hence the experiment throws little light on the effect of humidity on infection.

Eight 16-quart hampers of freshly harvested, sound carrots were inoculated by dipping them in a suspension of *Bacillus carotovorus* in beef bouillon. The carrots in four of the hampers were dried and one hamper was stored immediately at each of the relative humidities 95, 90, 80, and 70 per cent and at a temperature of 6.5° C. The four other hampers were first stored for 48 hours, without drying, at a temperature of 10° and a relative humidity of 95 per cent, and then placed, one at each humidity, together with the hampers of dried carrots. No infection occurred in the hampers of dried carrots even after 104 days of storage. In a wet hamper first exposed to a temperature of 10° and a relative humidity of 95 per cent for 48 hours and then stored at 6.5° and a relative humidity of 95 per cent, one root was found to be infected after 104 days of storage. Infection was also found on roots that had been given the same preliminary treatment and stored at a relative humidity of 70 per cent. No infection occurred in the wet roots at relative humidities of 80 and 90 per cent. These results are inconclusive as regards the effect of humidity on infection of carrots by *B. carotovorus* except to indicate, perhaps, that the preliminary wetting had some effect. Observations indicate that a high humidity favors infection at the temperatures at which infection normally occurs.

Varietal Susceptibility to Infection and Decay

Three types of experiments were employed in an attempt to measure the relative varietal susceptibility. (1) The roots were inoculated by the well method, one-half cubic centimeter of a suspension of *Bacillus carotovorus* in beef bouillon being used for each root. These roots were then stored at 20° C. The quantity of decay was determined by weight as previously described. (2) The roots in a 12-quart hamper of carrots were inoculated by placing at the center a root decaying with *B. carotovorus* and then storing them at 15°. (3) A 16-quart hamper of each variety was stored at harvest time at each of four temperatures, 0°–2°, 4.5°, 10°, and 15.5°, without treatment. Experiments of types 1 and 2 were repeated. In the first type of experiment the criteria used for the measurement of decay were the percentage of infection and the quantity of decay; in the second and third types the criteria used were the number and percentage of carrots infected.

The following 17 varieties of carrots have been found to be susceptible to decay by *Bacillus carotovorus*: Blanche à collet vert (hors terre), Blanche lisse demi-longue, Carter Early Market, Carter Long Forcing, Carter Nantes, Carter Red Elephant, Carter Scarlet Perfection, Carter Summer Favorite, Danvers Half Long, Rouge demi-longue de Danvers, Jaune obtuse du Doubs, Rouge à forcer Parisienne, Rouge demi-courte de Guérande, Rouge demi-longue d'Amsterdam, Rouge demi-longue de Chantenay, Rouge demi-longue Nantaise, and Rouge longue de Saint Valery.

Although there was considerable variation in infection and decay in the different varieties in a given experiment, the same variation was not maintained in others. If any variety showed greater susceptibility in contrast to other varieties, it was Blanche lisse demi-longue, and even in this case exceptions were found.

Botrytis Rot of Carrot (Gray-Mold Rot)

Occurrence

The attention given the Botrytis rot of carrot in literature is confined to a few reports of its occurrence in the field and in storage. It is common in storage, and it always occasions some loss whenever the roots are stored from two to three months at the usual temperatures (0° to 4.5° C.)—the longer the storage period, the greater the loss. As a rule the loss is not large, except in occasional instances after several months of storage.

The Pathogene

The Botrytis causing decay of carrot in transit and storage is of the *cinerea* type.[10] *Botrytis cinerea*, like *Bacillus carotovorus*, *Rhizopus tritici*, and *R. nigricans*, is nearly ubiquitous and is to some degree omnivorous. It is always present in carrot storage houses and will infect carrots at temperatures ranging from 0° to 7° C. and at relative humidities above 85 per cent, if the period of storage is long enough.

[10] The strain employed in the inoculation experiments was obtained from carrots and identified by Prof. H. H. Whetzel of Cornell University.

Factors Influencing Initiation of Infection

Effect of a nutrient medium

Some difficulty was experienced in obtaining uniform infection of carrots with *Botrytis cinerea* by artificial inoculation. Uniform infection was desired in order to measure quantitatively the effect of temperature on infection and decay. Inoculation by dipping the roots in a water spore suspension yielded as erratic results as did the storage of carrots at the various temperatures without inoculation. Infection resulting from inserting mycelium and spores into the roots by means of a scalpel was very limited and uncertain. Fairly uniform infection was obtained when the roots were inoculated in wells with a spore suspension in carrot decoction. Less infection occurred when roots were inoculated with a spore suspension in distilled water than when they were inoculated with a spore suspension in carrot decoction. (Table 20.) These results indicate that the presence of nutrient material other than the carrot tissue in the roots aids infection. Normal infection of carrots by *B. cinerea* in storage seems to be largely associated with and to follow the collapse of certain tissues. The points at which the pathogene gains entrance to the root are the fine tip end of the taproot, secondary roots (rarely), the crown, and wounds. Most of the infection takes place through the fine tip end of the taproot. Very little infection occurs during the first month or two at temperatures from 0° to 4.5° C. During this time some of the fine tip ends, the secondary roots, and some of the top tissues collapse and become necrotic, furnishing weakened or necrotic tissue upon which the pathogene can grow before establishing active relations with the host.

Effect of wounding

Aside from gaining entrance through the fine tip ends and the tops, Botrytis infection takes place almost exclusively through other wounds, especially through fresh wounds; rarely, if ever, through the uninjured skin; and seldom through secondary roots. The results recorded in Table 20 show that no infection occurred through rootlets or through uninjured skin when a spore suspension, either in carrot decoction or in distilled water, was used. Of the roots inoculated with a spore suspension in carrot decoction over fresh wounds, 75 per cent became infected; of those inoculated with a spore suspension in carrot decoction over old wounds, 27 per cent became infected; of those inoculated with a spore suspension in distilled water over fresh wounds, 40 per cent became infected; and of those inoculated with a spore suspension in distilled water over old wounds, none became infected.

In the absence of inoculation, severely wounded roots showed a slightly higher percentage (4 as compared with 0.8) of infection than unwounded roots (Table 21) when stored at the range of temperatures over which Botrytis commonly infects carrots. In this case one lot of unwounded carrots and one lot of roots that had been wounded by striking each one on the blunt edge of a wire basket were stored for 31 days at temperatures ranging from 2° to 15° C. The results (Tables 20 and 21) show that the uninjured skin is a barrier to infection and emphasize again the importance of careful handling of carrots in order to avoid wounding and consequent infection.

TABLE 20.—*Influence of wounds and of a nutrient medium on infection of carrots inoculated with Botrytis cinerea* [a]

Temperature (° C.)	Storage period	Infection of roots inoculated with spore suspension in—													
		Carrot decoction over—								Distilled water over—					
		Uninjured skin		Fresh wounds		Old wounds		Rootlets		Uninjured skin		Fresh wounds		Old wounds	
		Total	Infected	Total	Infected	Total	Infected	Total	Infected	Total	Infected	Total	Infected	Total	Infected
	Days	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*
8	21	5	0	5	5			5	0						
8	20	5	0	5	4	5	3			5	0	5	2	5	0
12	25	15	0	6	3	6	0	7	0						
Total		25	0	16	12	11	3	12	0	5	0	5	2	5	0

[a] The roots were inoculated by introducing a spore suspension into a glass tube 5 mm in diameter and 8 mm long, sealed over the infection court with vaseline. The fresh wounds were made by removing a small slice of tissue from one side of the root with a knife.

TABLE 21.—*Normal infection by Botrytis cinerea of wounded and unwounded carrots stored at various temperatures for 31 days*

Temperature (° C.)	Wounded roots			Unwounded roots		
	Total	Infected		Total	Infected	
	Number	*Number*	*Per cent*	*Number*	*Number*	*Per cent*
15	78	7	9	78	2	3
11.5	69	2	3	82	1	1
9.5	62	2	3	91	0	0
8	74	3	4	91	1	1
5	65	0	0	96	0	0
2	54	2	4	65	0	0
Total	402	16	4	503	4	8

TEMPERATURE RELATIONS

GROWTH OF THE PATHOGENE

To determine the influence of temperature on the growth of *Botrytis cinerea* ten 200-cc Erlenmeyer flasks, each containing 44 cc of carrot agar, were placed at each of 13 temperatures after each flask had been inoculated by introducing a platinum loop of a spore suspension of *B. cinerea* in sterile water into the agar at the center of the flask. No growth occurred at 35° C. in six days. (Table 22.) The highest temperature at which growth occurred was 32°; the maximum amount of growth occurred at 23.5°. As the temperature rose or fell below 23.5°, there was a rapid decline in the rate of growth. Some growth occurred at the lowest temperature employed, 0° to 1.5°. Growth has been observed in another experiment at a temperature of 0.5° to 1°. The cardinal temperatures for growth may be said to be approximately as follows: Maximum, 32° to 35°; optimum, 24°; and minimum, about 0°.

EXPERIMENTS ON INFECTION AND DECAY

Two types of experiments were employed in the study of the relation of temperature to infection and decay. Type 1 consisted of merely storing sound roots at various temperatures for the purpose of observing the infection that normally occurs. Type 2 consisted of a quantitative measurement of decay at various temperatures.

TYPE 1.—The data in Table 23 illustrate (1) the behavior of carrots stored at various temperatures at harvest time, and (2) the behavior of carrots stored at similar temperatures after previous storage at a temperature of about 0° C. In experiment 1, roots were taken directly from the field and stored at temperatures ranging from 1° to 31°. The periods of storage employed correspond very closely to the length of life of the roots. In experiment 2, the carrots were held in storage at a temperature of 0° to 2° from the first week in November until March 4, when they were stored at the various temperatures after the infected roots had been sorted out.

TABLE 22.—*Influence of temperature on the growth of Botrytis cinerea on carrot agar in Erlenmeyer flasks*

Temperature (° C.)	Average area of colonies after—				Temperature (° C.)	Average area of colonies after—			
	3 days	6 days	10 days	12 days		3 days	6 days	10 days	12 days
	Mm²	*Mm²*	*Mm²*	*Mm²*		*Mm²*	*Mm²*	*Mm²*	*Mm²*
35	0	0			17.5	1,158			
32	14				13	204	3,056		
28.5	1,393				11	133	2,333		
24	1,992				7	[a] 2	127	1,267	
23.5	2,608				6	0	102	870	
18.5	1,468				4.5	0	18	359	
					0–1.5	0	0	0	182

[a] Just started.

TABLE 23.—*Influence of temperature on infection of uninoculated carrots by Botrytis cinerea*

Experiment 1 [a]						Experiment 2 [b]					
Temperature (° C.)	Relative humidity	Storage period	Roots used	Roots infected		Temperature (° C.)	Relative humidity	Storage period	Roots used	Roots infected	
				Number	Per cent					Number	Per cent
	Per cent	*Days*	*Number*	*Number*	*Per cent*		*Per cent*	*Days*	*Number*	*Number*	*Per cent*
31	91	19	48	0	0	32	78	13	60	0	0
24.5	93	27	48	0	0	24.5	96	22	70	13	19
24	95	19	57	0	0	20	97	22	81	11	14
21.5	95	27	59	0	0	18	96	22	69	5	7
19	92	27	50	0	0	14.5	95	22	84	17	20
15.5	91	51	63	0	0	11.5	95	22	65	11	17
12.5	95	51	48	0	0	10	98	31	56	7	13
8	91	79	47	26	55	8	90	31	74	5	7
6.5	95	113	45	20	44	5	100	31	66	3	5
2	94	113	45	22	49	3	100	31	71	0	0
1	91	113	61	26	43						

[a] Newly dug roots exposed to various temperatures.
[b] Roots exposed to various temperatures after being stored at a temperature of 0° to 2° from the first week in November until Mar. 4.

The results in Table 23, experiment 1, represent what may be expected to happen normally if carrots are stored at various temperatures at harvest time, although infection often occurs at somewhat

higher temperatures and the percentage of infection varies considerably. As a rule, however, infection does not cover as wide a range of temperature in the storage of freshly dug carrots as in roots previously stored at a temperature of about 0° C. Infection in the freshly dug roots occurred only at temperatures of 1°, 2°, 6.5°, and 8°, whereas in the roots stored in March the range of infection extended from 5° to 24.5° and infection occurred in a somewhat shorter period of time. It should be stated, however, that 24.5° is the highest temperature at which infection has ever been obtained in the storage of untreated carrots. Generally, storage late in the season does not yield infection by Botrytis at quite so high a temperature.

The amount of infection by Botrytis at temperatures of 6° and above is often influenced by infection by other pathogenes.

The data recorded in Table 24 were obtained in connection with storage of carrots during four seasons in refrigerated rooms either 8 by 14 by 12 feet high, or 8 by 8 by 8 feet. The roots were stored at harvest time in bushel crates or in 16-quart hampers.

TABLE 24.—*Influence of temperature, humidity, and storage period on the normal infection of carrots by Botrytis cinerea during four seasons*

Storage season	Temperature	Relative humidity	Storage period	Roots used	Roots infected	
	° C.	*Per cent*	*Days*	*Number*	*Number*	*Per cent*
1924–25	10	80–96	102	1,288	84	7
	7	80–96	102	1,190	68	6
	4.5	75–83	102	1,029	60	6
	0–2	91	102	1,412	89	6
1925–26	10	75–85	121	437	112	26
	7	75–85	121	411	40	10
	0–2	90	121	886	49	6
	15.5	90	39	66	10	15
1926–27	10	80	99	58	2	3
	4.5	70	165	52	15	29
	0–2	90	131	199	105	[a] 53
	0–2	90	131	978	167	[b] 17
1927–28	12.5	91	50	121	0	0
	12.5	91	70	267	0	0
	4.5	84	50	134	2	1
	4.5	84	100	133	8	6
	4.5	84	150	124	58	47
	0–2	90	50	135	3	2
	0–2	90	100	129	6	5
	0–2	90	150	138	21	15
	0–2	86	50	132	3	2
	0–2	86	100	125	15	12
	0–2	86	150	134	25	19

[a] Single crate. [b] Average of several crates.

During the season of 1924–25 there was very little difference in the percentage of Botrytis rot present on carrots stored at any of the four temperatures employed. In 1925–26 the percentage of Botrytis rot increased with the rise in temperature. The variation in the length of the storage periods was such as to make the results at the different temperatures not comparable. At temperatures of 10° and 15.5° C. infection by Botrytis was complicated by infection by *Bacillus carotovorus* and forms of Fusarium. During the season of 1927–28 the percentage of infection after 50 to 100 days of storage was greater at 0° to 2° (if infection at the two humidities at this temperature is considered) than at 4.5°. After 150 days of storage the percentage of infection at 4.5° was 47 as compared with 19 per cent at 0° to 2°. No infection occurred at 12.5°, largely, it is believed, because of heavy infection by *B. carotovorus* and forms of Fusarium.

It can not be said on the basis of these results that an increase in temperature within the limits of those given will necessarily result in an increase in the amount of infection by Botrytis. In making this statement, due consideration is given the variation in the humidities involved in these experiments, which are not comparable. (The storage conditions in these experiments were utilized because they were the best available.) From the results at hand it would seem that humidity was not a limiting factor in these experiments. For instance, at a temperature of 0° to 2° there was very little difference in the amount of infection at 90 and at 86 per cent relative humidity, and contrary to what was expected, infection was greater at the lower humidity. There is often considerable variation in the amount of infection in individual containers stored under the same conditions. To illustrate, the percentage of infection in an individual crate stored at 0° to 2° during the season of 1926–27 was 53, while the average in several crates was 17. The factor involved in this variation is not known. The results obtained during the season of 1927–28 show the relation of time to infection at temperatures of 0° to 2° and 4.5°. Very little infection occurred during the first 50 days. The percentage increased more rapidly during the succeeding intervals of 50 days.

The results in Table 25 were obtained in connection with experiment 1, Table 23, and illustrate the effects of various periods of time on infection at 1°, 2°, 6.5°, and 8° C. In 19 days no infection had occurred in roots stored at 6.5° and 8° and none in 27 days at 1° and 2°. There was a progressive increase in the percentage of infection at each temperature with the lapse of time, except that there was only 44 per cent infection at 6.5° after 113 days of storage as compared with 53 per cent in 79 days at the same temperature. Infection by Penicillium, which obscured the previous infection, was responsible for the apparent decrease in infection by Botrytis.

It should be stated that the percentages of Botrytis infection given, especially at temperatures of 0° to 2° C. in the last three tables, are not necessarily translatable into equal values of losses, especially during the shorter periods of storage. Most of the infection takes place through the broken tip ends of the taproots, and any penetration of the thicker portion of the roots is included in the count. In cases where infection has merely begun to decay the thickened portion of the root, practically the entire root is unimpaired for consumption.

TYPE 2.—As has been stated, some difficulty was experienced in obtaining uniform infection of carrots by Botrytis. Quantitative data under such circumstances are rather uncertain. The problem was further complicated at the higher temperatures because of contamination by such pathogenes as Rhizopus and *Bacillus carotovorus*.

The data given in Table 26 were obtained from one experiment and in the main are representative of the results secured in two other experiments.

The carrots were thoroughly washed and inoculated by introducing an equal quantity of a spore suspension of *Botrytis cinerea* in carrot decoction into a well 2 mm in diameter, penetrating to the center of the thickest portion of the root. They were stored in wire baskets, at the various temperatures shown in Table 26, in infection chambers provided with ventilation. The relative humidity was above 90 per cent at each temperature.

TABLE 25.—*Influence of storage period on infection of uninoculated carrots by Botrytis cinerea at various temperatures*

Temperature (° C.)	Relative humidity	Roots used	Roots infected after—			
			27 days	51 days	79 days	113 days
	Per cent	*Number*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
8	91	47	4	25	55	
6.5	95	45	2	24	53	44
2	94	45	0	9	31	40
1	91	61	0	8	25	39
1	91	[a] 152–187		6		25

[a] These carrots were stored in crates in a storage room, whereas the remaining lots were stored in hampers in infection chambers. One crate (containing 152 roots) was inspected after 51 days and one (containing 187 roots) was inspected after 113 days.

TABLE 26.—*Influence of temperature on infection and decay of carrots inoculated with Botrytis cinerea* [a]

[5 roots used in all cases]

Temperature (° C.)	Storage period	Roots infected		Average area of lesions	Temperature (° C.)	Storage period	Roots infected		Average area of lesions
	Days	*Number*	*Per cent*	*Mm²*		*Days*	*Number*	*Per cent*	*Mm²*
30	4	0	0	0	9.5	9	5	100	273
28	4	0	0	0	7	9	1	20	[b]
24.5	4	4	80	234	5	9	0	0	0
22.5	4	5	100	344	7	17	5	100	410
20	4	5	100	240	5	17	5	100	111
18.5	4	5	100	240	2	17	0	0	0
12	4	0	0	0	2	42	3	60	474
12	9	5	100	654					

[a] The relative humidity of the storage chambers was above 90 per cent.
[b] Just started.

The highest temperature at which infection occurred in this experiment was 24.5° C. (Table 26.) In another experiment infection was obtained at 28°. Both temperatures are somewhat below the maximum for growth of *Botrytis cinerea* (32°). (Table 22.) This might well be expected, since infection by Botrytis at the higher temperatures is somewhat uncertain. (Table 23, experiment 1.) The greatest amount of decay after four days occurred at 22.5°, an optimum temperature corresponding closely with that for growth of this pathogene on culture media. After nine days there was 654 mm² decay at 12°, 273 mm² at 9.5°, a trace at 7°, and none at 5°. The amount of decay declined during this period as the temperature rose above or fell below 22.5°. Considerable decay occurred at 2° in 42 days in three out of five roots. Infection has occurred at temperatures as low as 0°.

The maximum and minimum temperatures for infection based on all temperature experiments conducted are approximately 24.5° to 28° and 0° C., respectively. The optimum for the rate of decay is about 23°.

Influence of Humidity on Infection and Decay

The data available are too few to permit one to draw definite conclusions regarding the influence of humidity on infection of carrots by *Botrytis cinerea*. In one experiment (Table 27) freshly dug carrots were employed. Eight hampers of carrots were inoculated by dipping

the roots in a spore suspension of the pathogene, the other four hampers (checks) were stored, without treatment, at the relative humidities of 95, 90, 80, and 70 per cent, respectively, and at a temperature of 6.5° C. Four hampers of the inoculated roots were poured out on a clean floor and dried by means of an electric fan. The roots were then returned to the dry hampers. One hamper each of dried and undried (wet) inoculated carrots was stored with each of the checks. The length of the storage period was 104 days.

TABLE 27.—*Influence of humidity on the infection of carrots inoculated with Botrytis cinerea and stored for 104 days at a temperature of 6.5° C.*

Relative humidity (per cent)	Inoculated roots						Untreated roots		
	Wet			Dried					
	Total	Infected		Total	Infected		Total	Infected	
	Number	*Number*	*Per cent*	*Number*	*Number*	*Per cent*	*Number*	*Number*	*Per cent*
95	139	9	6	107	8	7	106	10	9
90	127	9	7	118	10	8	100	6	6
80	126	2	2	108	2	2	132	11	8
70	124	2	2	129	0	0	107	0	0

There was no significant difference in the percentage of infection in either the inoculated or uninoculated roots at relative humidities of 90 or 95 per cent. As the relative humidity fell from 90 to 80 per cent there was a considerable drop in the percentage of infection in the inoculated roots, but not in the checks. There was no infection in the dried inoculated roots or checks at a relative humidity of 70 per cent. At this humidity there was only 2 per cent infection in the wet inoculated roots; the same percentage of infection was obtained in wet roots at 80 per cent relative humidity. These results indicate that a relative humidity of 70 per cent and possibly of 80 per cent is unfavorable to infection. This humidity, however, is also unfavorable for the storage of carrots.

VARIETAL SUSCEPTIBILITY TO INFECTION AND DECAY

In addition to the 14 varieties of carrot listed in Table 28, the following 4 varieties have been found susceptible to infection and decay by *Botrytis cinerea:* Blanche à collet vert (hors terre), Blanche lisse demi-longue, Jaune obtuse du Doubs, and Carter Summer Favorite.

Two types of experiments were conducted to determine the relative susceptibility of the different varieties. In one type the roots were artificially inoculated, and in the other infection was dependent on the inoculum normally present on the roots.

INOCULATION EXPERIMENTS

In each of two experiments, 20 carrots of each variety were washed in soapy water and dried in the laboratory. By means of a hypodermic needle, 0.2 cc of a spore suspension of *Botrytis cinerea* in carrot decoction was then introduced into a well 2 mm in diameter and 2 cm deep in the thickest diameter of the root. The roots were stored

in wire baskets at the same level in a room 8 by 8 by 8 feet, at a temperature of 12° C. The length of the storage period in the first experiment was 12 days and in the second 16 days.

The criteria used in measuring the relative susceptibility of the several varieties were: (1) Percentage of roots infected, (2) average diameter of the lesions based on the total number of inoculations, and (3) average diameter of the lesions that developed in each variety. The diameters of the lesions were obtained from the cross sections of the roots cut in two directly lengthwise through the well. The first two criteria are measures of the ability of the fungus to infect and the amount of decay occasioned, and the third the measure of its ability to penetrate the tissue after it has established relations with the host.

TABLE 28.—*Susceptibility of 14 varieties of carrot to decay when inoculated with Botrytis cinerea* [a]

Variety	Roots infected			Average diameter of lesions based on—					
				Number of roots			Number of lesions		
	Experiment 1	Experiment 2	Mean	Experiment 1	Experiment 2	Mean	Experiment 1	Experiment 2	Mean
	Per cent	*Per cent*	*Per cent*	*Mm*	*Mm*	*Mm*	*Mm*	*Mm*	*Mm*
Carter Early Market	95	80	88	21	23.8	22.2	20	19	19.5
Carter Long Forcing	33	30	32	15.4	11.5	13.6	5.1	3.5	4.3
Carter Nantes	90	90	90	22.1	25	23.5	19.8	22.3	21.7
Carter Red Elephant	95	89	92	20.6	22.6	21.5	19.5	20	19.8
Carter Scarlet Perfection	52	80	66	17.4	20.4	19.1	9.1	16.4	12.6
Danvers Half Long	------	90	90	------	21	21	------	19	19
Rouge demi-longue de Danvers	90	------	90	19.5	------	19.5	19.5	------	19.5
Rouge à forcer Parisienne	85	90	88	21.8	28.8	25.3	18.5	25.1	21.8
Rouge demi-courte de Guérande	100	90	95	22.4	25.5	24	22.4	23	23.9
Rouge demi-longue d'Amsterdam	100	84	92	18.9	21.4	20	18.9	18	18.5
Rouge demi-longue de Chantenay	85	55	70	21.4	23.4	22.2	18.2	12.9	15.5
Rouge demi-longue de Saint James	84	------	84	17.3	------	17.3	14.6	------	14.6
Rouge demi-longue Nantaise	100	90	95	19.4	22	20.6	19.4	19.8	19.6
Rouge longue de Saint Valery	80	100	90	22.2	25.8	24	17.8	25.8	21.6

[a] Duration of first experiment, 12 days; of second experiment, 16 days.

The percentage of infection in this case is no doubt a more accurate index to susceptibility than the diameter of the lesions because of certain difficulties arising from the size of the roots in the several varieties and the variation in the relative thickness of the core and cortical tissue, which affect the rate of penetration and hence the diameter of the lesions. The cortical tissue, at times at least, seems to be more resistant to decay than the core and tends to limit the degree of decay. Any variation in the amount of cortex and core would therefore affect the amount of decay. The size of the roots would also influence this variation, for the advance of decay would be inhibited earlier in the smaller roots than in the large ones. Only one variety (Table 28), Carter Long Forcing, consistently showed a considerable degree of resistance when judged by all three criteria in both experiments. Two other varieties, Carter Scarlet Perfection and Rouge demi-longue de Chantenay, showed a somewhat lower percentage of infection than the other 15 varieties. Most of the varieties were highly susceptible.

EXPERIMENTS WITH UNINOCULATED ROOTS

A 16-quart hamper of each of the varieties listed in Table 29 was taken directly from the field (October 28, 1926) and stored at each of four temperatures (0 to 2°, 4.5°, 10°, and 15.5° C.) in storage rooms 8 by 14 by 11 feet high. The hampers were placed at the same level in each room. Hampers stored at 0° to 2° were placed between two shelves in a space just high enough for them. A second lot of each variety [11] was stored in bushel crates at 0° to 2° in the same room as the hampers. The crates were stacked five high, in adjacent tiers.

TABLE 29.—*Susceptibility of 17 uninoculated varieties of carrot to infection by Botrytis cinerea*

Variety	Percentage of roots infected at indicated temperature (° C.), relative humidity (per cent), and storage period (days) when—				
	Stored in 16-quart hampers				Stored in bushel crates (0°–2°; 90 per cent; 131 days)
	15.5°; 90 per cent; 39 days	10°; 80 per cent; 99 days	4.5°; 70 per cent; 165 days	0°–2°; 90 per cent; 131 days	
Blanche à collet vert (hors terre)	32	0	6.5	35.2	5.8
Blanche lisse demi-longue	0	9.5	18.1	51.4	34.4
Carter Early Market	11.3	1.7	12.3	25.8	18.8
Carter Long Forcing	13.1	0		28.2	
Carter Nantes	17	0	16.7	27.2	9.5
Carter Red Elephant	8.9	0	16.7	22.1	18.8
Carter Scarlet Perfection	22.7	2.6	27.7	29.1	6.7
Carter Summer Favorite	9.8	2.2	10	14.8	19.6
Danvers Half Long	15.2	3.5	28.8	52.8	17.1
Jaune obtuse du Doubs	8.3	0	32.1	34.7	14.5
Rouge demi-longue de Danvers	13.4	0	7.5	26.9	14.8
Rouge à forcer Parisienne	15.5	4.1	25.6	25.9	25.4
Rouge demi-courte de Guérande	2.9	6.2	16.1	21.7	8.2
Rouge demi-longue d'Amsterdam	4.2	1.7	10.4	21.5	20.9
Rouge demi-longue de Chantenay	27	3	15.5	21.9	15.3
Rouge demi-longue Nantaise	5	2.3	10.7	14.2	2.2
Rouge longue de Saint Valery	14.3	4.7	6.8	17.4	1.9

In all varieties, except Carter Summer Favorite, the percentage of infection at 0° to 2° C. was higher in the hamper lot than in the crate lot, and in most varieties considerably higher. Just why there should have been so marked a difference in the two lots is not entirely clear. The relative humidity readings were taken in the open part of the room and represent the humidity of the air surrounding the crates rather than that surrounding the hampers. It is possible, because of the position of the hampers between the shelves and their closeness to each other, that the humidity was higher and that there was less ventilation in the hampers than in the crates. These factors may have increased the amount of infection. In any case it is probable that the difference in the percentage of infection in the two lots was not wholly accidental.

The results at the different temperatures can not be compared directly, desirable though such a comparison would be, because of the differences in the humidity of the storage rooms and in the duration of the storage periods.

The data at 10° and 15.5° C. show considerable variation in the percentage of infection, and in a number of cases a complete absence

[11] Carter Long Forcing was not stored at 4.5° or in crates at 0° to 2°.

of infection. A large part of the variation was due to bacterial and Penicillium decay. For instance, in the case of the Blanche lisse demi-longue variety, all the roots stored at 15.5° were decayed by *Bacillus carotovorus*. At a temperature of 4.5°, infection by Penicillium was rather severe in some cases and may have influenced the amount of Botrytis infection.

At temperatures of 0° to 2° and 4.5° C., in both hamper and crate storage, there was considerable variation in the percentage of infection in the different varieties, but the variation in the case of any one variety under the three conditions was not striking or consistent enough to make it possible to draw very definite conclusions as to variation in resistance. Carter Long Forcing, instead of showing the greatest resistance among the varieties with which it is compared in Table 28, shows less resistance than certain other varieties.

As a result of these experiments it may at least be inferred that all the varieties tested are susceptible to infection and decay by Botrytis and that the small amount of resistance shown by any one variety does not give it much advantage over the other varieties.

DISCUSSION

TEMPERATURE AS A GOVERNING FACTOR IN THE INFECTION AND DEVELOPMENT OF STORAGE DISEASES OF CARROTS

Aside from such diseases as Sclerotinia soft rot and black rot (*Alternaria radicina* Meier, Drechs., and Eddy), which directly or indirectly have their origin in the field, diseases that develop in the storage house are governed largely by temperature, provided that the relative humidity of the storage room is high enough to prevent shriveling.

If carrots are stored at harvest time at temperatures ranging from 1° to 37° C., results similar to those presented in Table 30 are usually obtained. The percentage of infection and the temperature limits of the various diseases may be expected to vary somewhat. It may be added as supplementary to these data that carrots stored at temperatures above 37° often show evidence of injury aside from infection, manifested in the blackening and killing of the tissue. The length of life of the roots at these temperatures is only one or two days, and Penicillium invades most of the roots and sometimes all of them. In addition to Penicillium, a very sticky bacterium is found particularly associated with the injured roots. The diseases that normally develop in roots stored at temperatures from 0° to 37° are Rhizopus soft rot, bacterial soft rot, Fusarium rot, Penicillium rot, and Botrytis rot. Unidentified lesions also develop sometimes, and upon very rare occasions *Rhizoctonia solani* Kühn infects carrots at temperatures near 0°. Because of the overlapping of the temperature ranges of infection by some of the pathogenes, the amount of decay produced by any one pathogene was influenced by that produced by another.

Figure 1 illustrates the distribution of infection by the various pathogenes at the different temperatures during the different periods of time. A, B, C, D, and E represent the separate distribution of infection by Rhizopus, *Bacillus carotovorus*, Fusarium, Penicillium, and *Botrytis cinerea*, respectively, and F is a composite graph of the distribution of infection by the five pathogenes. The graphs are not intended to convey any idea of the quantity of decay present but merely to show the occurrence of infection at the different temperatures

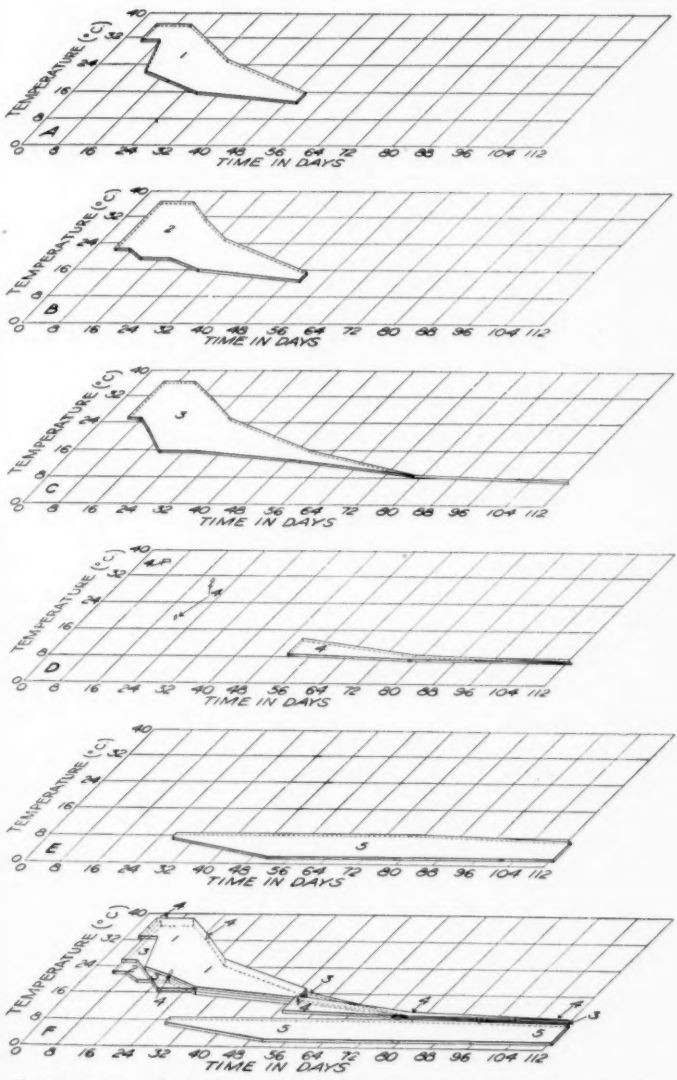

FIGURE 1.—Influence of temperature and time on the occurrence of infection by the organisms that normally decay carrots when stored at a range of temperatures extending from 1° to 36.5° C.: A, Rhizopus; B, *Bacillus carotovorus;* C, Fusarium; D, Penicillium; E, *Botrytis cinerea;* of A, B, C, D, and E, combination F, represented by 1, 2, 3, 4, and 5, respectively

TABLE 30.—*Influence of temperature on normal infection of carrots by Rhizopus soft rot, bacterial soft rot, Penicillium rot, Botrytis rot, and Rhizoctonia rot after varying periods of time*

Temperature (° C.)	Relative humidity	Roots used	Percentage of roots infected by indicated pathogene after—																			
			4 days	5 days				6 days				8 days			12 days			19 days			
			Rhizopus soft rot	Rhizopus soft rot	Bacterial soft rot	Fusarium rot	Penicillium rot	Rhizopus soft rot	Bacterial soft rot	Fusarium rot	Penicillium rot	Rhizopus soft rot	Bacterial soft rot	Fusarium rot	Rhizopus soft rot	Bacterial soft rot	Fusarium rot	Rhizopus soft rot	Bacterial soft rot	Fusarium rot	Penicillium rot
	Per cent	*Number*																				
36.5	93	43	26	44	2	9	2	56	5	14	2	70	7	21	81	7	9				
31	91	48	2	4	6	2	0	4	6	2	0	4	6	2	15	21	44	19	25	40	0
24.5	93	48	0	0	2	4	0	0	2	4	0	0	2	4	0	17	8	4	54	35	0
24	95	57													0	54	4	0	95	2	0
21.5	95	59	0	0	2	0	0	0	2	0	0	0	2	0	2	8	3	3	15	63	0
19	92	50	0	0	0	0	0	0	0	0	0	0	0	0	0	6	0	2	14	60	2
15.5	91	63	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0
12.5	95	48	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
8	91	47	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
6.5	95	45	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	94	45	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1	91	61	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Temperature (° C.)	Relative humidity	Roots used	Percentage of roots infected by indicated pathogene after—															
			27 days				51 days					79 days			113 days			
			Rhizopus soft rot	Bacterial soft rot	Fusarium rot	Botrytis rot	Rhizopus soft rot	Bacterial soft rot	Fusarium rot	Penicillium rot	Botrytis rot	Fusarium rot	Penicillium rot	Botrytis rot	Fusarium rot	Penicillium rot	Botrytis rot	Rhizoctonia rot
	Per cent	*Number*																
36.5	93	43																
31	91	48																
24.5	93	48	8	67	25	0												
24	95	57																
21.5	95	59	8	42	46	0												
19	92	50	10	36	48	0												
15.5	91	63	8	5	59	0	8	17	71	0	0							
12.5	95	48	0	0	0	0	4	15	54	6	0							
8	91	47	0	0	0	4	0	0	0	6	25	6	23	55				
6.5	95	45	0	0	0	2	0	0	0	0	24	0	2	53	5	11	44	0
2	94	45	0	0	0	0	0	0	0	0	9	0	0	31	0	0	40	0
1	91	61	0	0	0	0	0	0	0	0	8	0	0	25	0	0	39	2

during different periods of time. The graphs are incomplete in that they do not show the infection that occurs above 36.5° and below 1° C. Furthermore, the periods of time given do not always correspond at the lower limits to the time required for the initial infection to occur at a given temperature. The graphs do, however, give some idea of the distribution of infection, the conditions of temperature and time in which infection is absent, and the extreme length of life of the carrots at temperatures above 6.5°. The recording of data at these temperatures was discontinued only when the deterioration of the roots was such as to preclude their consumption. The time required for the roots to reach this state of deterioration is approximately represented by the upper receding line of each graph. This line marks the discontinuance of recording data at the various temperatures.

The data presented in Table 30 and Figure 1 show that if carrots are stored at or near 1° C. the limiting disease originating in storage is Botrytis rot. Fortunately its development is very slow. The other diseases become limiting factors only when roots are stored or transported at temperatures unfavorable for the holding of carrots for reasons other than infection. Table 31 shows that sprouting begins in roots held at temperatures of 19°, 21.5°, 24.5°, and 31° in 5 days. No sprouting occurred at 36.5° in 12 days. Sprouting began at 15.5° and 12.5° in 12 days, at 8° in 19 days, and at 6.5° in 27 days, whereas at 1° and 2° no sprouting was apparent in 113 days. It is evident, therefore, that decay by Rhizopus, *Bacillus carotovorus*, Fusarium, and Penicillium occurs, as a rule, only when deterioration from other causes has set in. The sprouts themselves soon deteriorate at temperatures of 10° and above, because of infection by bacteria and fungi. As a result of this latter infection, the roots become discolored and slimy.

Development of Disease at Common Storage Temperatures

Roots of the Danvers Half Long variety were stored four different years at various ranges of temperatures the extreme limits of which were 0° to 2° and 15.5° C. (Table 32.) The range probably covers that usually employed in common storage, and the results illustrate the losses that might be expected provided Sclerotinia soft rot was not present.

Bacterial soft rot was consistently present in considerable quantity at temperatures of 10° C. and above. The losses below 10° were very slight, and only in one season, 1926–27, did bacterial soft rot (0.2 per cent) occur at 0° to 2°.

Some black rot occurred each season, but the amount present had no relation to temperature. Moreover, it was not always present under all the conditions of storage, although it develops readily under all these conditions, indicating that the pathogene is not as ubiquitous as *Bacillus carotovorus* and Rhizopus.

TABLE 31.—*Influence of temperature on sprouting of carrot roots*

Temperature (° C.)	Time required for roots to begin sprouting	Temperature (° C.)	Time required for roots to begin sprouting	Temperature (° C.)	Time required for roots to begin sprouting	Temperature (° C.)	Time required for roots to begin sprouting
	Days		*Days*		*Days*		*Days*
36.5	(a)	21.5	5	12.5	12	2	(b)
31	5	19	5	8	19	1	(b)
24.5	5	15.5	12	6.5	27		

[a] None in 12 days. [b] None in 113 days.

TABLE 32.—*Development of diseases in the Danvers Half Long variety of carrot under various conditions of storage during four seasons*

Storage season	Temperature	Relative humidity	Storage period	Roots used	Roots infected	Percentage of roots infected with—							Roots infected at tip ends [a]	Sound roots plus roots infected at tip ends
						Bacterial soft rot	Black rot	Fusarium rot	Penicillium rot	Rhizopus rot	Unidentified decay	Botrytis rot		
	°C.	*Per cent*	*Days*	*Number*	*Per cent*								*Per cent*	*Per cent*
1924–25	10	80–96	102	1,288	60	6	1	0.1	0	0	0	7	46	86
	7	80–96	102	1,190	41	.8	.4	0	0	0	0	6	34	93
	4.5	75–83	102	1,029	20	0	.6	0	0	0	0	6	13	93
	0–2	91	102	1,412	17	0	.2	0	0	0	0	6	11	94
1925–26	10	75–85	121	437	59	13	.7	0	2	.2	.4	26	17	58
	7	75–85	121	411	35	.7	.7	0	4	.3	0	10	19	84
	0–2	90	121	886	24	0	4	0	0	0	0	6	14	90
1926–27	15.5	90	39	66	68	33	7	33	6	0	0	15	14	46
	10	80	99	58	64	19	4	0	2	0	0	3	0	36
	4.5	70	165	52	58	0	0	0	25	0	0	29	0	42
	0–2	90	131	199	88	0	.1	0	0	0	0	53	35	47
	0–2	90	131	978	51	.2	0	0	.9	.1	.4	17	32	81
1927–28	12.5	91	50	121	81	44	.7	21	0	0	16	0	0	19
	12.5	91	70	267	94	77	0	10	0	0	6	0	0	6
	4.5	84	50	134	16	0	0	1	0	0	0	3	12	96
	4.5	84	100	133	28	4	3	1	0	0	0	11	12	84
	4.5	84	150	124	60	0	0	0	0	0	0	47	10	50
	0–2	90	50	135	5	0	2	0	0	0	0	2	3	98
	0–2	90	100	129	20	0	1	0	0	0	0	5	13	93
	0–2	90	150	138	31	0	0	0	0	0	0	15	15	84
	0–2	86	50	132	3	0	0	0	0	0	0	2	1	98
	0–2	86	100	125	22	0	0	0	0	0	0	12	10	88
	0–2	86	150	134	43	0	0	1	0	0	0	19	23	80

[a] The decay under this heading is confined to the thin portion at the root tip and does not affect the market value of the carrot.

Fusarium rot was present each season except one, but the amount of loss that it occasioned varied greatly. Although its development is influenced largely by temperature, its presence or absence seems to be governed by some other factor. In one instance (1927–28) infection occurred at a temperature of 0° to 2° C.; otherwise infection by Fusarium has never been observed at this temperature and very little has been found at temperatures below 8°.

Penicillium was present during two seasons and occurred at as low a temperature as 0° to 2° C. Its presence or absence at these low temperatures seems to be largely governed by some factor other than temperature.

A large percentage of the carrots were infected each season at the fine tip ends of the roots. The infection so listed in the table never reached the thickened portion of the root and did not materially

affect the market value of the carrots, because the diseased tips would usually be broken off in the course of preparation for the market. From the standpoint of infection, roots showing the percentages given in the last column of Table 32 might be regarded as fit for market. Because of germination and infection of the tops and discoloration of the roots, the carrots stored at temperatures above 4.5° C. were unfit for market, whereas those stored at 0.2° and 4.5° C. are regarded as still unimpaired in market value.

Numerous isolations were made from the infected tip ends. The organisms obtained varied greatly. Among them were *Botrytis cinerea, Bacillus carotovorus, Alternaria radicina,* and forms of Fusarium and Penicillium. Because of the small size of these fine tip ends and the fact that the decay involves the entire cross section, one is almost as likely to get a contaminating organism as the pathogene. At temperatures between 0° and 5° C., most of these infections ultimately lead to Botrytis decay.

The results for the season 1927–28 (Table 32) illustrate the progress of decay during storage at three temperatures and in the case of 0°–2° C. at two humidities. Lower humidities were employed at each temperature, but the roots were so badly shriveled from loss of water that the results are not submitted.

At 12.5° C. very few roots remained sound after 50 days of storage. At 4.5° the loss was only 4 per cent in the first 50 days, but by the end of 150 days only 50 per cent remained marketable. At 0° to 2° there was very little difference in the losses at the two humidities, and 80 and 84 per cent of the roots were marketable after 150 days.

TABLE 33.—*Infection of the Danvers Half Long variety of carrots stored for various periods at 0° to 2° C. during five seasons*

Storage season	Relative humidity	Storage period	Roots used	Roots infected	Percentage of roots infected with—							Roots infected at tip ends [a]	Roots marketable
					Bacterial rot	Black rot	Fusarium rot	Penicillium rot	Rhizopus soft rot	Unidentified decay	Botrytis rot		
	Per cent	*Days*	*Number*	*Per cent*								*Per cent*	*Per cent*
1923–24	91	201	1,727	57	0	0.2	0	0	0	0	16	41	84
1924–25	91	102	1,412	17	0	.2	0	0	0	0	6	11	94
1925–26	90	161	886	24	0	4	0	0	0	0	6	14	90
1926–27	85	131	978	50	.2	.1	0	.9	.1	.4	17	31	81
1927–28	85	50	131	3	0	0	0	0	0	0	2	.8	98
	85	100	125	22	0	0	0	0	0	0	12	10	88
	85	150	134	43	0	0	.8	0	0	0	19	23	80

[a] The decay in this case is confined to thin portions of root tip and does not affect the marketable value of the carrot.

The results recorded in Table 33 give the percentage of losses due to different diseases of the Danvers Half Long variety during five different years of storage at 0° to 2° C. and the progress of decay during the season 1927–28. The duration of the storage ranged from 50 to 201 days and the losses for the season from 2 to 20 per cent. Most of the losses were due to Botrytis rot and included roots that showed any infection whatever of their thickened portion.

The results of the year 1927–28, it is believed, give a fair picture of the possible losses that might be expected after various periods of storage, because the greatest total loss was incurred during this season. It is true that in two of the seasons the storage period was shorter, but in two of them it was longer.

Varietal Susceptibility to Decay at Common Storage Temperatures

The results recorded in Table 34 were obtained from one season's storage of 17 varieties of carrots at temperatures of 0° to 2°, 4.5°, 10°, and 15.5° C. in 16-quart hampers and at 0° to 2° in crates.

The roots in hampers were stored on the same level at each temperature, whereas those in crates were stored in mass. The results are representative in the main of those obtained during two other seasons. An exception is the development of bacterial soft rot at a temperature of 0° to 2° C.; in fact, bacterial soft rot has never been found at any other time at this temperature during nine years of storage of the Danvers Half Long variety.

TABLE 34.—*Influence of certain temperatures in storage on normal infection of 17 varieties of carrots* [a] [b]

Variety	Percentage of carrots infected at indicated temperature (° C.) and humidity (per cent)																									
	By Bacillus carotovorus					By Fusarium					By Penicillium					By Botrytis cinerea					By Alternaria radicina				
	15.5° 90	10° 80	4.5° 70	0°-2° 90	0°-2° 90	15.5° 90	10° 80	4.5° 70	0°-2° 90	0°-2° 90	15.5° 90	10° 80	4.5° 70	0°-2° 90	0°-2° 90	15.5° 90	10° 80	4.5° 70	0°-2° 90	0°-2° 90	15.5° 90	10° 80	4.5° 70	0°-2° 90	0°-2° 90
Blanche à collet vert (hors terre)	32	14	0	1	1	0	0	0	0	0	0	24	3	0	0	32	0	7	35	6	0	3	0	0	0
Blanche lisse demi-longue	100	81	0	0	5	0	0	0	0	0	0	5	23	0	0	0	10	18	51	34	0	0	5	0	0
Carter Early Market	68	22	5	2	.7	0	36	5	0	0	0	7	5	0	0	11	2	12	26	19	2	3	15	2	4
Carter Long Forcing	69	64	----	8	----	4	11	----	0	0	2	2	----	0	----	13	0	----	28	----	0	0	----	.5	----
Carter Nantes	36	11	0	0	2	0	25	3	0	0	0	4	11	0	1	17	0	17	27	10	0	0	10	1	1
Carter Red Elephant	69	32	0	1	3	2	23	4	0	0	2	15	10	0	1	9	0	17	22	19	0	4	0	.6	.5
Carter Scarlet Perfection	48	8	0	3	3	0	36	3	0	0	0	8	11	0	1	23	3	28	29	7	0	0	0	0	0
Carter Summer Favorite	69	67	3	6	5	0	13	1	0	0	0	2	3	0	0	10	2	10	15	20	2	0	1	2	.9
Danvers Half Long	33	19	0	0	.2	0	33	0	0	0	6	2	25	0	.9	15	4	29	53	17	0	7	4	0	.1
Rouge demi-longue de Danvers	29	29	0	3	0	0	13	0	0	0	29	24	30	0	0	13	0	8	27	15	0	0	5	.8	0
Jaune obtuse du Doubs	79	63	0	2	0	0	11	0	0	0	0	4	7	0	0	8	0	32	35	15	4	4	4	0	.9
Rouge à forcer Parisienne	43	69	0	0	3	14	12	6	0	0	4	2	17	0	0	16	4	26	26	25	2	2	9	0	1
Rouge demi-courte de Guérande	88	59	0	.9	.9	0	19	0	0	0	0	3	0	0	0	3	6	16	22	8	0	0	7	4	3
Rouge demi-longue d'Amsterdam	67	47	0	0	2	2	17	0	0	0	0	3	19	0	1	4	2	10	22	21	2	2	4	3	2
Rouge demi-longue de Chantenay	41	42	0	.8	1	0	24	5	0	0	5	3	0	0	0	27	3	18	22	15	0	3	18	0	.6
Rouge demi-longue Nantaise	50	30	0	.8	0	0	16	0	0	0	0	2	2	0	0	5	2	11	14	2	0	0	0	0	0
Rouge longue de Saint Valery	53	21	0	2	1	0	21	0	0	0	6	12	34	0	2	14	5	7	17	2	0	2	0	0	1

Variety	Percentage of carrots infected at indicated temperature (° C.) and humidity (per cent)													Percentage of sound roots plus roots infected at tip ends only, at indicated temperature (° C.) and humidity (per cent)				
	By unknown cause					At tip ends only				Total [c]								
	15.5° 90	10° 80	4.5° 70	0°–2° 90	0°–2° 90	15.5° 90	10° 80	4.5° 70	0°–2° 90	15.5° 90	10° 80	4.5° 70	0°–2° 90	0°–2° 90	15.5° 90	10° 80	4.5° 70	0°–2° 90	0°–2° 90
Blanche à collet vert (hors terre)	0	0	3	0	1	0	0	3	22	64	41	16	58	33	36	59	87	64	92
Blanche lisse demi-longue	0	0	0	0	0	0	0	0	17	100	95	46	68	53	0	5	55	49	60
Carter Early Market	0	0	0	0	7	10	0	1.5	24	90	70	43	53	53	19	31	63	70	76
Carter Long Forcing	0	0		0		0	0		17	88	77		53		12	23		64	
Carter Nantes	0	0	3	0	0	9	0	3	21	62	42	46	49	25	47	59	57	72	83
Carter Red Elephant	0	0	2	0	0	0	0	2	35	82	75	35	59	42	18	26	67	76	76
Carter Scarlet Perfection	0	0	3	0	0	0	0	0	25	71	54	44	57	20	20	42	56	68	90
Carter Summer Favorite	0	0	4	0	0	6	0	10	41	86	85	32	63	50	20	15	78	78	74
Danvers Half Long	0	0	0	0	.4	14	0	0	35	68	64	58	87	51	45	36	42	47	81
Rouge demi-longue de Danvers	0	0	0	0	0	4	0	5	33	76	[d] 71	48	64	35	29	29	58	69	85
Jaune obtuse du Doubs	0	0	0	0	0	0	0	4	33	92	81	43	69	51	8	19	57	63	85
Rouge à forcer Parisienne	0	0	11	0	0	3	0	0	39	81	90	68	65	57	22	10	32	74	70
Rouge demi-courte de Guérande	3	0	3	0	0	0	0	0	36	94	88	24	62	49	6	13	74	74	88
Rouge demi-longue d'Amsterdam	0	0	0	0	.5	4	0	15	31	79	71	48	55	54	25	29	67	76	74
Rouge demi-longue de Chantenay	0	0	0	0	.6	0	0	15	40	73	76	55	63	57	26	24	60	77	82
Rouge demi-longue Nantaise	0	0	0	0	.5	8	0	4	16	63	51	17	31	22	45	49	87	85	97
Rouge longue de Saint Valery	0	2	2	0	0	10	0	2	31	84	63	45	51	30	27	37	57	80	84

[a] Of the two columns of data under 0°–2° in each group, the first refers to roots stored in 16-quart hampers, the second to roots stored in crates.
[b] The storage periods were: At 15.5°, 39 days; at 10°, 99 days; at 4.5°, 165 days; and at 0°–2°, 131 days.
[c] Totals refer to all preceding data. Small discrepancies are due to the fact that the total infection was not calculated but was recorded from observation.
[d] Five per cent were infected with Rhizopus.

The relative susceptibility of the different varieties to bacterial soft rot and Botrytis has already been discussed, but it remains to point out that there is considerable variation in the percentage of infection with both diseases in the two lots stored at 0° to 2° and that they are not parallel. It is believed that this variation is normal and not due to the difference in container or position.

There is some variation in the percentage of infection of the different varieties at a particular temperature by Fusarium and Penicillium, but this variation is obviously affected by infection by the other pathogenes; nor is the variation always in the same direction at the other temperatures. Taking into consideration the total infection and the results in the last column, it is quite evident that no one variety is distinctly more resistant to disease than another.

Kristofferson (7), working with the Parisian carrot, Guérande, two strains of Nantes, and two of Saint Valery, not only found a difference in susceptibility to "winter rot," but discovered also that this susceptibility was correlated with the invert sugar content. For instance Guérande, with an invert sugar content of 4.53 per cent, showed 84.6 per cent rot, as compared with Saint Valery, strain B, with an invert sugar content of 2.43 per cent, which showed only 5 per cent rot.

These results are difficult of interpretation, (1) because the storage temperatures and other conditions of storage are not given and (2) because "winter rot" is rather a general term. At any rate, no such marked difference in susceptibility was found in these experiments, in these or other varieties tested. If the invert sugar content governs the susceptibility of a particular lot of carrots in any way, this susceptibility can not be expected to remain constant from year to year, for Hasselbring (5) found a greater variation in the invert sugar content of the same variety for different seasons than among the different varieties for the same season.

EFFECT OF HUMIDITY ON SHRIVELING

Carrot roots of all varieties are very susceptible to drying and shriveling, and the range of humidities at any particular temperature at which it is possible to maintain a desired turgor is relatively narrow.

At relative humidities of 90 and 95 per cent and a temperature of 6.5° C. (Table 35)[12] all the roots remained firm during 104 days of storage, whereas there was considerable shriveling at both 70 and 80 per cent relative humidity (much more at 70 than at 80 per cent) after both 47 and 104 days of storage. At 70 per cent relative humidity there was a marked increase in shriveling as the storage period was prolonged. The data show a smaller percentage of shriveling at 80 per cent relative humidity after 104 days of storage than after 47 days. The numbers used in the two counts may have made some difference; otherwise there is no evident explanation of this discrepancy.

Although no data are available on the relation of humidity to shriveling at temperatures near 0° C., which is the most desirable temperature for the storage of carrots, experience shows that the relative humidity should range from 90 to 95 per cent.

[12] The data recorded in Table 35 were obtained from the same experiment as those given in Tables 6 and 27.

TABLE 35.—*Influence of humidity on shriveling of carrots stored at 6.5° C.*

Relative humidity (per cent)	Storage period	Roots used	Roots shriveled		Relative humidity (per cent)	Storage period	Roots used	Roots shriveled	
	Days	*Number*	*Number*	*Per cent*		*Days*	*Number*	*Number*	*Per cent*
95	47	726	0	0	95	104	723	0	0
90	47	596	0	0	90	104	622	0	0
80	47	694	116	17	80	104	220	31	14
70	47	710	367	52	70	104	253	206	81

SUMMARY AND CONCLUSIONS

The market-inspection certificates issued by the United States Department of Agriculture on the 214 cars of topped carrots inspected in the United States during the years 1922 to 1927, inclusive, show the following average percentages of decay: 6.3 per cent from Sclerotinia soft rot, 2.6 per cent from Rhizopus soft rot, 1.2 per cent from bacterial soft rot, and 2.9 per cent from Botrytis rot.

Since a large number of these cars were inspected for condition and grade independent of decay, it is believed that these figures give some indication of the losses and their causes in the carrots inspected and a suggestion of the losses that generally occur in transit.

Although the uninjured skin of carrot roots is an effective barrier against infection by *Sclerotinia sclerotiorum*, yet because of the discontinuity of the skin due to old wounds, secondary roots, and the broken tip ends of the taproots, infection takes place readily whenever the pathogene is present.

Fresh wounding, although not indispensable to infection, increases the amount.

Sclerotinia sclerotiorum was found to grow on carrot agar at temperatures ranging from 0.9° to 32.5° C. No observations were made at temperatures below 0.9°. The maximum amount of growth occurred at 24°.

Infection of carrots by *Sclerotinia sclerotiorum* has been obtained at temperatures ranging from 0°–1° to 28° C. The greatest amount of decay was obtained at 23°. The difference between the optimum for growth of *S. sclerotiorum* on agar and that for decay of carrots by the same organism is believed to be due to the difference in the temperatures employed in the two cases. Although it is not practicable to store carrots below the temperature limit for infection by *S. sclerotiorum*, the processes of infection and decay proceed very slowly at or near 0°. Since the Sclerotinia soft rot has its origin directly or indirectly in the field, its control should begin in the field.

Roots dipped in a suspension of mycelium of *Sclerotinia sclerotiorum* and agar in water and stored while wet at relative humidities of 80 and 90 per cent at a temperature of 6.5° C. became infected. The percentage of infection was much higher at 90 than at 80 per cent relative humidity.

Roots inoculated in the same manner as the foregoing and dried with an electric fan were stored at relative humidities of 95, 90, 80, and 70 per cent at a temperature of 6.5° C. Those stored at 90 and 95 per cent relative humidity became infected, whereas those stored at 70 and 80 per cent did not. The percentage infected at 95 per cent relative humidity was higher than that at 90 per cent.

By storing dry roots, contaminated but not infected with *Sclerotinia sclerotiorum*, at a temperature of 0° and relative humidities ranging from 85 to 90 per cent, it is believed possible greatly to inhibit infection.

Fourteen varieties of carrots have been found readily susceptible to infection by *Sclerotinia sclerotiorum*. No marked difference in susceptibility was observed among them.

Rhizopus tritici and *R. nigricans* are the only species of Rhizopus obtained from a large number of isolations from carrots subjected to a variety of temperatures and other storage conditions. Although it is possible that other species may decay carrots under special conditions, they do not do so normally under Washington conditions.

The extreme temperature limits at which infection of carrots by Rhizopus has been obtained in uninoculated roots are 0° to 2° and 44° C. Infection is always relatively heavy and occurs within a period of four days at temperatures above 30°. At temperatures below 30° infection is always relatively light, and the time required for it to occur is much longer. Below 20° infection is erratic in its occurrence, often not occurring at all during the marketable life of the roots and never in large amounts. In only two tests has infection been observed at temperatures below 12°, namely, at 0° to 2° and 10° in the season of 1926–27 and at 7° and 10° in the season of 1925–26 (Table 32 and unpublished data), and then it was very limited.

The time required for Rhizopus infection to develop in uninoculated roots increased with the lowering of the temperature below 36.5° C. Only 4 per cent occurred at 12.5° in 51 days.

The extreme temperature limits at which infection of uninoculated carrots by *Rhizopus tritici* has been obtained are 19° and 44° C.; in roots inoculated by the well method infection has been obtained at 5°.

The extreme temperature limits at which infection of uninoculated carrots by *Rhizopus nigricans* has been obtained are 0° to 2° and 19° C. Infection by this pathogene in roots inoculated by the well method has occurred at 28.5°, and possibly higher. The upper temperature limit for infection by this pathogene has not been accurately determined, because of complications produced by infection by *Rhizopus tritici*.

Infection by *Rhizopus nigricans* is normally rare and small in amount, indicating that carrots are highly resistant to attack by this pathogene.

The optimum temperature for decay of carrots inoculated by the well method with *Rhizopus tritici* was about 33.5° C., and for those inoculated with *Rhizopus nigricans* the optimum temperature was about 28°. Roots inoculated by being dipped in a spore suspension of either pathogene, or both, may show a small increase in the percentage of infection within certain temperature limits and a lower limit of infection. However, such is not always the case, nor is the effect ever marked.

Wounding generally tends to increase the percentage of infection by Rhizopus and to drop the lower limit of infection, but the effect is not marked nor is wounding indispensable to infection.

Seventeen varieties of carrots were found to be susceptible to infection and decay by *Rhizopus nigricans* when the roots were inoculated by the well method. During three years of normal storage of

these varieties at temperatures of 0° to 2° C. the only infection by *R. nigricans* observed, aside from that reported for the Danvers Half Long variety, occurred during the season 1926–27 at a temperature of 10° in the Rouge demi-longue de Danvers variety. The slight and rare infection in Danvers Half Long and Rouge demi-longue de Danvers and the absence of infection in all the other varieties indicate that all varieties are normally resistant to decay by *R. nigricans* at temperatures from 0°–2° to 15.5°.

Sixteen varieties of carrot, dipped in a spore suspension of *Rhizopus tritici* and stored at 30° C. or inoculated by the well method and stored at 23°, were very susceptible to infection and decay. No marked differences in susceptibility to this fungus were found.

That *Bacillus carotovorus* is constantly associated with carrots is indicated by the fact that a certain percentage of roots will invariably become infected if stored at temperatures ranging from 20° to 25° C., if the relative humidity is high (90 to 95 per cent).

Wounding was found to increase slightly the percentage of infection at temperatures ranging from 17.5° to 24° C.; however, it was not essential to infection, a large percentage of infection occurring in its absence.

The extreme temperature limits at which infection of inoculated carrots by *Bacillus carotovorus* has been observed are 0° to 2° and 36.5° C. In only 1 out of 9 seasons of normal storage of the Danvers Half Long variety and in 1 out of 4 seasons of storage of several other varieties has bacterial soft rot been observed at a storage temperature of 0° to 2°. Infection has occurred in only 1 out of 3 seasons of normal storage of several varieties at 4.5°. Storage trials with the Danvers Half Long variety at temperatures near 4.5° have failed to yield infection by *Bacillus carotovorus*. In roots that were sound when stored, very little infection has ever been observed at temperatures below 10°.

There was little, if any, apparent difference in the time required for infection to occur in carrots stored at temperatures ranging from 21.5° to 36.5° C. As the temperature was lowered from 21.5° to 12.5° the time required for infection to develop increased, at 12.5° no infection occurring in 27 days and 15 per cent in 51 days.

The inclusion in the storage stock of carrots decaying with *Bacillus carotovorus* insures a more uniform distribution of the decay over a wider range of temperatures than occurs in the storage stock of roots free from such contamination.

In carrots inoculated by the well method the maximum temperature at which infection occurred within three days was 29° C.; the optimum temperature for decay was 24.5°; and the minimum temperature for infection in seven days was 5°.

Taking into consideration the three types of experiments employed, the optimum temperature for decay is about 25° C.

The results regarding the influence of humidity on infection of carrots by *Bacillus carotovorus* are too incomplete and indecisive to make it possible to draw any conclusions from them.

Seventeen varieties of carrots have been found readily susceptible to infection and decay by *Bacillus carotovorus*. Although some variation in the percentage of infection and in the quantity of decay has been found in the different varieties in a given experiment, this variation

was not always paralleled in a second experiment, and differences in susceptibility have not been sufficiently consistent to give any variety an outstanding position in regard to susceptibility or resistance. The Blanche lisse demi-longue variety may be slightly more susceptible than the others.

The uniformity with which Botrytis rot develops when carrots are stored for long periods at temperatures ranging from 0° to 5° C. indicates that *Botrytis cinerea* is always present in carrot storage houses.

Infection of carrots by *Botrytis cinerea* is aided by the presence of nutrient media as used in the well method of inoculation. Normal infection seems to be associated with the collapse of certain tissues such as tops, secondary roots, wounded areas, and the fine tip ends of taproots. Most of the infection occurs in the last-mentioned way. Aside from the infection that occurs through secondary roots, the fine tip ends of taproots, and the tops, infection takes place almost exclusively through wounds, more readily through fresh wounds than old ones, and rarely if ever through the uninjured skin.

The cardinal temperatures for the growth of *Botrytis cinerea* on culture media are approximately as follows: Maximum, 32° to 35° C.; optimum, 24°; and minimum, about 0°.

Botrytis rot in carrots taken directly from the field and stored at various temperatures is usually confined to temperatures below 15° C. If carrots are placed at higher temperatures after having been stored at a temperature near 0° for a time, the range may be extended to higher temperatures.

Infection of carrots by Botrytis takes place slowly in roots taken directly from the field and stored at temperatures ranging from 0° to 2° C.; that is, the percentage of infection is relatively small during the first 50 days and decay is, as a rule, in its initial stage. Infection and decay increase slowly as the storage period extends beyond 50 days.

The widest range of temperatures at which *Botrytis cinerea* infection has been obtained on carrots extends from 0°–2° to 24.5° C., as compared with a temperature range extending from 0° to 32° for growth of *B. cinerea* on carrot agar. The maximum amount of decay on carrots was obtained at 22.5°, which is very close to the optimum temperature (23.5°) obtained for growth of the pathogene on carrot agar. The difference may be accounted for by the difference in temperatures employed in the two experiments.

In carrots taken directly from the field and stored without inoculation at a temperature of 6.5° C. for 104 days there was very little difference in the percentage of infection at relative humidities of 80, 90, and 95 per cent. There was a drop in the percentage of infection as the relative humidity fell from 90 to 70 per cent. There was no infection at a relative humidity of 70 per cent in uninoculated and dried inoculated roots and only 2 per cent in roots stored when wet, indicating that 70 per cent relative humidity inhibits infection. This humidity, however, is too low for the proper storage of carrots, since it causes them to shrivel.

Eighteen varieties of carrots have been found to be susceptible to infection and decay by *Botrytis cinerea*. No consistent differences in their susceptibility to this fungus were found.

If carrots free from contamination by *Sclerotinia sclerotiorum* are stored at temperatures ranging from 0° to 40° C. and at relative humidities of 90 per cent and above for a period covering the market

life of the carrots at the various temperatures, the following diseases may be expected to develop: Rhizopus soft rot, bacterial soft rot, Fusarium rot, Penicillium rot, and Botrytis rot. The percentage of roots infected and the temperature limits at which infection by the various pathogenes takes place will vary somewhat with the stock stored. The amount of decay produced by one pathogene often influences the amount produced by another.

In a certain experiment in which carrots were stored at temperatures ranging from 1° to 36.5° C. (Table 30), the temperature limits within which decay occurred were as follows: Rhizopus soft rot, 12.5° and 36.5°; bacterial soft rot, 12.5° and 36.5°; Fusarium rot, 6.5° and 36.5°; Botrytis rot, 1° and 8°; and Penicillium rot, 6.5° and 36.5°. Penicillium rot occurred at only one temperature (19°) between 12.5° and 36.5°, and mostly at 6.5° to 12.5°, inclusive.

The market life of carrots, with reference to the development of diseases, increases as the temperatures are lowered below 36.5° C.

A factor other than infection and decay that greatly limits the life of carrots at temperatures from 6.5° to 31° C. is sprouting. No sprouting occurred on roots stored at 36.5° for 12 days. Sprouting occurred at 31°, 24.5°, 21.5°, and 19° in 5 days; at 15.5° and 12.5° in 12 days; at 8° in 19 days; and at 6.5° in 27 days. No sprouting occurred at 1° and 2° in 113 days.

The diseases that developed in the Danvers Half Long variety at one temperature or another during four different years of storage at temperatures ranging from 0°–2° to 15.5° C. were: Bacterial soft rot, black rot, Fusarium rot, Penicillium rot, Rhizopus soft rot, and Botrytis rot. This range of temperatures coincides approximately with the limits one might expect to find in common storage.

The losses during five different years of storage of the Danvers Half Long variety at temperatures fluctuating between 0° and 2° ranged from 2 to 20 per cent. The relative humidities varied during the several seasons from 85 to 91 per cent and the storage periods from 50 to 201 days.

A survey of the diseases that developed in 17 varieties of carrots stored at temperatures ranging from 0°–2° to 15.5° C. during the season 1926–27 and in most of the varieties during four other seasons showed that all the varieties were susceptible to Fusarium rot and to Penicillium rot in addition to the diseases discussed hitherto. No marked difference in susceptibility was found.

No shriveling occurred in carrots stored at a temperature of 6.5° C. and at relative humidities of 90 and 95 per cent, whereas considerable shriveling occurred at relative humidities of 70 and 80 per cent.

The environmental conditions regarded as most favorable for storage of carrots are a temperature of 0° C. and a relative humidity of 90 to 95 per cent.

LITERATURE CITED

(1) Boyle, C.
1921. STUDIES IN THE PHYSIOLOGY OF PARASITISM. VI. INFECTION BY SCLEROTINIA LIBERTIANA. Ann. Bot. [London] 35:[337]–347, illus.

(2) Harter, L. L., and Weimer, J. L.
1921. A COMPARISON OF THE PECTINASE PRODUCED BY DIFFERENT SPECIES OF RHIZOPUS. Jour. Agr. Research 22:371–377, illus.

(3) HARTER, L. L., and WEIMER, J. L.
1922. DECAY OF VARIOUS VEGETABLES AND FRUITS BY DIFFERENT SPECIES OF RHIZOPUS. Phytopathology 12:[205]–212.
(4) ——— WEIMER, J. L., and ADAMS, J. M. R.
1918. SWEET-POTATO STORAGE ROTS. Jour. Agr. Research 15:337–368, illus.
(5) HASSELBRING, H.
1927. CARBOHYDRATE TRANSFORMATIONS IN CARROTS DURING STORAGE. Plant Physiol. 2:225–243.
(6) JONES, L. R.
1901. A SOFT ROT OF CARROT AND OTHER VEGETABLES, CAUSED BY BACILLUS CAROTOVORUS. Vt. Agr. Expt. Sta. Ann. Rpt. (1899–1900) 13:299–332.
(7) KRISTOFFERSON, K. B.
1921. ON THE RELATION BETWEEN SUGAR CONTENT AND WINTER ROT IN THE GARDEN CARROTS. Bot. Notiser 1921:149–163.
(8) LAURITZEN, J. I.
1926. INFECTION AND TEMPERATURE RELATIONS OF BLACK ROT OF SWEET-POTATOES IN STORAGE. Jour. Agr. Research 33:663–676, illus.
(9) ——— and HARTER, L. L.
1923. SPECIES OF RHIZOPUS RESPONSIBLE FOR THE DECAY OF SWEET-POTATOES IN THE STORAGE HOUSE AND AT DIFFERENT TEMPERATURES IN INFECTION CHAMBERS. Jour. Agr. Research 24:441–456.
(10) ——— and HARTER, L. L.
1925. THE INFLUENCE OF TEMPERATURE ON THE INFECTION AND DECAY OF SWEETPOTATOES BY DIFFERENT SPECIES OF RHIZOPUS. Jour. Agr. Research 30:793–810, illus.
(11) ——— and HARTER, L. L.
1926. THE RELATION OF HUMIDITY TO INFECTION OF THE SWEET-POTATO BY RHIZOPUS. Jour. Agr. Research 33:527–539, illus.
(12) RAMSEY, G. B.
1924. SCLEROTINIA INTERMEDIA N. S. A CAUSE OF DECAY OF SALSIFY AND CARROTS. Phytopathology 14:[323]–327, illus.
(13) ———
1925. SCLEROTINIA SPECIES CAUSING DECAY OF VEGETABLES UNDER TRANSIT AND MARKET CONDITIONS. Jour. Agr. Research 31:597–632, illus.
(14) WEISS, F., LAURITZEN, J. I., and BRIERLEY, P.
1928. FACTORS IN THE INCEPTION AND DEVELOPMENT OF FUSARIUM ROT IN STORED POTATOES. U. S. Dept. Agr. Tech. Bul. 62, 36 p., illus.
(15) WRIGHT, R. C., and TAYLOR, G. F.
1923. THE FREEZING TEMPERATURES OF SOME FRUITS, VEGETABLES, AND CUT FLOWERS. U. S. Dept. Agr. Bul. 1133, 8 p.

THE SPOILAGE OF DRESSED DUCKS BY SLIMINESS[1]

By W. L. MALLMANN

Research Assistant Bacteriologist, Department of Bacteriology, Michigan Agricultural Experiment Station

INTRODUCTION

A select and extensive market has been created for the sale of what the trade designates as green ducks, squab ducklings, or Long Island ducks. These ducks are from 5 to 10 weeks of age and weigh from 3 to 5 pounds. The immaturity of their tissues renders them very soft and tender, with the result that they spoil rather rapidly, much more so than mature carcasses.

When squab ducklings are shipped in crushed ice or ice water, very little spoilage results; but when they are shipped moist in containers which permit them to come in contact with the air, they become slippery, particularly beneath the wings. In extreme cases the entire carcass is covered with slime so thick that it is difficult to pick up the duck with the hands. Accompanying the slime formation is a markedly offensive odor. In the early stages decomposition is purely superficial and washing in a solution of sodium carbonate will remove all odor and slime. The skin, however, has a roughened appearance, which renders the duck unfit for market. The internal organs and other tissues do not show any decomposition or odor even in pronounced cases of sliminess.

Slipperiness will develop in from one to two days even when carcasses are kept constantly at ice-box temperature (50° F.). Carcasses stored in meat-market ice-cooled refrigerators spoil with marked regularity. Mechanical refrigeration at a temperature of 40° F. prevents sliminess but causes a drying out of the skin, which is accompanied by discoloration and cracking. The resulting product is as unmarketable as the slimy ducks, owing to the objectionable appearance of the carcasses.

This type of spoilage first came to the attention of the writer when a Michigan packing plant began shipping ducks in individual cartons direct to the consumer. This plant had previously shipped its entire output in crushed ice without any evidence of slime formation. At the time that sliminess was observed in ducks shipped in paper cartons, those shipped in crushed ice were free from this trouble. All the ducks were dressed in the same plant at the same time under identical conditions, the only difference in the preparation was in the mode of shipping. Further investigation revealed the fact that sliminess is of frequent occurrence in squab-duckling packing plants and, furthermore, that this condition never occurs when the ducks are shipped in crushed ice.

[1] Received for publication Dec. 21, 1931; issued July, 1932. Journal article No. 86 (n. s.) from the Michigan Agricultural Experiment Station.

EXPERIMENTAL STUDIES

Bacteriological examinations made from slimy ducks revealed several spore-forming bacilli. The predominating organism was a large, nonmotile, Gram-positive, spore-forming bacterium with a large capsule. The organism has all the characteristics of *Bacillus mesentericus* except motility. It appears to be either an undescribed form or a nonmotile variant of *B. mesentericus*. It was obtained in practically pure culture from the ducklings examined.

In the killing plant cultures were made from all the tanks and tables in or upon which the ducks were handled. The same organism was isolated from each of the following places: The scalding tank (140° F.), plumping tank (212°), chilling tank (50°), ice-water tank, dressing tables, floor, feet of live ducks entering the plant, and shelves in ice chest. The organism was not found in the water supply used at the killing plant.

To check further the significance of the organism as a causative factor in producing sliminess, five live ducklings from this plant were dressed at the college poultry plant, sterilized containers being used for scalding and cooling. The college dressing plant was completed shortly before this experiment was conducted, so all the equipment was new. In order to be sure that all contamination was excluded, the tanks were carefully cleaned with a strong solution of sodium hypochlorite, and 100 parts per million of chlorine in the form of sodium hypochlorite was added to the scalding water to destroy any organisms that might be present on the ducks. The method of dressing was exactly the same as that used at the duck-packing plant. After the ducks were dressed and cooled, two were immersed for five minutes in a suspension of the encapsulated organism. The remaining three were kept as controls. All the ducks were then placed in paper cartons and immediately stored in an ice-cooled refrigerator at a temperature of 50° F. A duck dressed in the packing plant was stored in the same refrigerator. Each day for a period of eight days the ducks were removed from the refrigerator and examined for slime formation. Within two days the duck dressed at the plant was too slimy to be marketable. On the sixth day the inoculated ducks were slippery under the wings, but the control ducks were free from spoilage. On the eighth day the inoculated ducks were decidedly slimy, the slime then covering the entire carcass. An offensive odor was present. One of the controls was slightly slippery under the wings, but the other two were still quite normal The same organism was recovered from all the ducks that showed evidence of slime formation. The results obtained clearly indicate that the organism isolated is a causative factor in the production of sliminess and that the source of contamination is the utensils used in the dressing and cooling processes. Thus it appeared that a clean plant might solve the problem. Accordingly, means by which this might be accomplished were considered.

The use of a chemical disinfectant appeared to be the best procedure, since the organisms apparently survive boiling, as indicated by their isolation from the plumping tank at a temperature of 212° F. A

disinfectant that would act in the presence of excessive quantities of organic matter was necessary; also it should have a low toxicity, as it would be undesirable to add any substance that would injure the food value of the duck.

Sodium hypochlorite was finally selected because it meets these requirements and because of its adaptability and low cost. Since much dirt and contamination enter the scalding tanks, it was believed that any attempt to use continuous disinfection in these tanks would not be feasible. Instead, a system of disinfection in the final ice-water storage tanks was attempted. In these tanks sodium hypochlorite in doses of 100 parts per million of available chlorine was added. Two tanks were used; one was treated with neutral sodium hypochlorite and the other with an alkaline sodium hypochlorite. The dressed ducks were stored in these tanks for approximately 24 hours, after which they were removed, packed in cartons, and stored in an ice-cooled refrigerator. Untreated ducks were similarly stored as controls. The results of this experiment are presented in Table 1.

TABLE 1.—*Results of disinfecting dressed ducks in chlorinated ice water*

Duck No.	Treatment	Condition of carcass after—	
		6 days	10 days
1	None	Decidedly slimy and discolored	Poor.
2	do	do	Do.
3	Neutral sodium hypochlorite	Slimy and discolored	Fair.
4	Alkaline hypochlorite	Excellent	Best.
5	do	Slightly slimy on legs only	Good.
6	do	Slimy and discolored	Fair.

At the end of six days, only one duck (No. 4) that was treated with neutral sodium hypochlorite was really marketable as a product of the highest quality. The control ducks (untreated) were unmarketable at the end of six days. At the end of 10 days all the ducks were unmarketable; however, the treated ducks were in better condition than the untreated ones. The experiment demonstrated that treatment with sodium hypochlorite in ice water will extend the keeping period but that it does not eliminate ultimate spoilage by slime formation.

To determine the efficiency of hypochlorite for killing the organisms causing sliminess, a series of laboratory experiments was made in which a technic was used similar to that employed in determining the phenol coefficient of a disinfectant. Temperatures of 10°, 20°, and 37° C. were used with exposures of 1, 5, 10, 30, 60, and 120 minutes. The culture was a 48-hour agar slant containing spores. Two series of tests were made, one with 0.1 cc of culture suspension and the other with 0.01 cc of suspension. Three strengths of hypochlorite were used, namely, 25, 50, and 100 parts per minute of available chlorine. The results are presented in Table 2. Only the data for 0.1 cc of culture suspension are given, since the results with 0.01 cc were the same.

TABLE 2.—*Killing power of sodium hypochlorite as determined on a 0.1 c c culture suspension of the organism causing sliminess in dressed ducks*

Available chlorine	Temperature of exposure	Negative or positive results after exposure of—					
		1 minute	5 minutes	10 minutes	30 minutes	60 minutes	120 minutes
P. p. m.	° *C.*						
25	10	+	+	+	+	+	+
50		+	+	+	+	+	+
100		+	+	+	+	+	+
25	20	+	+	−	−	−	−
50		+	−	−	−	−	−
100		−	−	−	−	−	−
25	37	+	−	−	−	−	−
50		−	−	−	−	−	−
100		+	−	−	−	−	−

The data indicate that sodium hypochlorite in ice water at all strengths tested was ineffective during the periods of exposure used. At 20° and at 37° C. the chlorine was comparatively active, causing complete destruction of all vegetative cells and spores in five minutes or less.

In a later examination of slimy ducks, three other organisms were isolated—a noncapsule-forming spore producer, a coccus, and a nonspore-forming rod. These organisms were selected because the odor of the cultures was identical with that found on the slimy ducks. Their resistance to chlorine was studied. The results are presented in Table 3.

TABLE 3.—*Killing power of sodium hypochlorite as determined on three microorganisms found on slimy ducks*

Culture No.[a]	Available chlorine	Temperature of exposure	Control	Negative or positive results after exposure of—					
				1 minute	5 minutes	10 minutes	15 minutes	20 minutes	30 minutes
	P. p. m.	° *C.*							
1	50	22	+	−	−	−	−	−	−
6			+	−	−	−	−	−	−
10			+	−	−	−	−	−	−
1	25		+	+	+	−	−	−	−
6			+	+	−	−	−	−	−
10			+	−	−	−	−	−	−
1	50	7	+	−	−	−	−	−	−
6			+	−	−	−	−	−	−
10			+	−	−	−	−	−	−
1	25		+	−	−	−	−	−	−
6			+	−	−	−	−	−	−
10			+	−	−	−	−	−	−

[a] Culture No. 1, a noncapsule-forming, spore-forming bacillis; No. 6, a Gram+micrococcus; No. 10, a Gram+nonspore-forming rods.

The three organisms were readily killed by chlorine in ice water in concentrations of 25 p. p. m. of available chlorine. The fact that one of the organisms was a spore former indicates that chlorine does have a decided germicidal value at low temperatures, and that in spite of its inability to destroy all the capsulated spore formers present, it can reduce to a marked degree the amount of contamination carried on the surface of the duck.

Although chlorine in the quantities used had no effect on the appearance of the ducks, a careful examination was made of ducks

after 24 hours' immersion in 100 p. p. m. of available chlorine to determine whether the chlorine had impaired their value as food. The treated ducks showed no injurious effects from the treatment. Nevertheless, it was thought that a minimum amount of chlorine should be used. To determine what the minimum should be, experiments were conducted with small amounts of residual chlorine. When 3.5 p. p. m. of available chlorine was used, all the culture tubes showed growth up to 30 minutes, the longest period of exposure employed. However, there was a decided reduction in the number of bacteria as evidenced by the bacterial counts. Apparently the most effective results were obtained with a minimum of 25 p. p. m. of available chlorine, which was sufficient for complete disinfection.

A second method of control was attempted in which the ducks were immersed in a disinfecting or antiseptic solution of sufficient strength to assure a retention of the antiseptic substance on the duck until it reached the consumer. The disinfectant used had to be one free from objectionable odor and taste. Sodium hypochlorite, sodium borate, sodium salicylate, and sodium chloride were tried. The sodium hypochlorite when used in strong solutions produced a medicinal odor in the paper carton that would be objectionable to the consumer. The sodium borate and sodium salicylate were not sufficiently antiseptic to retard putrefaction materially; hence these compounds were discarded. Dipping the ducks in a saturated solution of sodium chloride, however, eliminated the formation of slime. In this procedure the chemical is not used up by combining with the organic matter, as is the case with sodium hypochlorite. Instead, as the moisture evaporates from the surface of the duck, the salt becomes more concentrated and hence more active as an inhibiting agent. Sodium chloride, moreover, has no objectionable features as a food preservative.

To determine the concentration of salt necessary to inhibit the growth of the bacteria, different quantities of sodium chloride were added to plain nutrient broth into which the various cultures were planted by adding a loopful of a 24-hour broth suspension. The results are presented in Table 4.

TABLE 4.—*Inhibitive action of sodium chloride upon the growth of microorganisms causing slime formation in dressed ducks*

Microorganism No.	Control	Negative or positive results when using a concentration of salt of—		
		2 per cent	5 per cent	10 per cent
1	+	+	+	−
6	+	+	+	−
10	+	+	+	−

The above table shows that a concentration of 10 per cent of sodium chloride is necessary to suppress the development of the bacteria. That the action is purely inhibitive and not germicidal was demonstrated by making plate counts from these tubes at various intervals up to 20 minutes. Although counts were made from salt concentrations of 2, 5, and 10 per cent, only the last are presented, as similar results were obtained with the 2 and 5 per cent concentrations. The data are shown in Table 5.

TABLE 5.—*Effect of a 10 per cent solution of sodium chloride on the count of certain microorganisms causing slime formation in dressed ducks*

Microorganism No.	Control count	Count after exposure of—				
		1 minute	5 minutes	10 minutes	15 minutes	20 minutes
1	133	261	201	200	192	149
6	186	253	256	219	246	251
10	235	323	245	266	261	254

It will be observed that no reduction occurred in the number of bacteria. In fact, there was an apparent increase, probably owing to the repeated shaking of the tubes at the time of sampling which caused a dispersion of clumps of bacteria.

DISCUSSION

As the data indicate, two methods of controlling slipperiness in squab ducklings may be used, namely, sanitation and preservation. The experiments demonstrate that the use of clean, sterile utensils with clean, sterile, scalding, plumping, and chilling waters will result in a product that develops sliminess very slowly, the period of delay being sufficient for the usual marketing. However, the maintenance of such sanitary conditions is not practicable in the usual killing plant and is prohibitive in cost, so it becomes necessary to use chemical preservatives along with economical and practical sanitary measures. It has been demonstrated that a disinfection of the carcass with sodium hypochlorite in the final ice-water storage tank materially lessens the amount of contamination and thus delays spoilage. A final dipping of the finished product in a concentrated salt solution results in covering the carcass with a thin film of salt brine that inhibits the growth of the slime-producing bacteria and thus prevents spoilage. In practice the treatment of the ducks by immersing in ice water containing sodium hypochlorite, followed by dipping in saturated salt brine just previous to packing for shipment, has given very satisfactory results. It may be that the antiseptic action of the salt brine is sufficient and that the addition of sodium hypochlorite to the storage tank is unnecessary.

SUMMARY

Slipperiness in squab ducklings is caused by bacteria.

Four microorganisms were isolated from slimy ducklings. The chief microorganism involved in slipperiness is a spore-forming capsulated bacillus closely resembling *Bacillus mesentericus*. This organism was found on all utensils and exposed surfaces in the killing and dressing rooms of the duck-packing plant investigated.

Slime formation was induced by inoculating ducklings with pure culture of this organism.

The addition to the storage tanks of 100 p. p. m. of available chlorine in the form of sodium hypochlorite delayed the formation of slime.

Dipping the dressed ducklings in saturated salt brine stopped the formation of slime.

EFFECT OF FERTILIZERS ON THE CHLORINE CONTENT OF THE SAP OF CORN PLANTS[1]

By N. A. PETTINGER[2]

Associate Agronomist, Virginia Agricultural Experiment Station

INTRODUCTION

In a recent paper (*14*)[3] the writer reported some preliminary results of an investigation which showed that the composition of the sap of corn (*Zea Mays* L.) plants was markedly affected by the addition of fertilizers to the soils on which the plants were grown. At the time these results were reported, the sap samples had been analyzed for nitrate nitrogen, total phosphorus, and total potassium only. In an effort to explain certain data obtained in the corn-sap studies now under way at the Virginia station, it seemed desirable to determine whether the various sap samples contained large, medium, or small amounts of some of the fertilizer constituents that are usually given minor consideration in the use of fertilizers. Accordingly, the sap samples were analyzed for the materials of interest. The purpose of the present paper is to present the data from these analyses that concern mainly the effect of chlorine-carrying fertilizers on the chlorine content of the sap of corn plants as found in a 4-year study under field conditions.

MATERIALS AND METHODS

The plants from which sap was extracted for this investigation were grown on the "rotation with fertilizer" plots at the Virginia Agricultural Experiment Station, Blacksburg, Va. Since a complete description of these plots and their management has been given in a previous report (*14*), only the more salient features will be repeated here. The experiment was started in 1909 on a Hagerstown silt loam soil of moderate fertility, and embraced four series of 13 plots each. These four series of plots, designated as A, B, C, and D, carry in rotation the following crops: First year, corn; second year, wheat; third and fourth years, hay (mammoth clover, redtop, and timothy). The kinds and the combinations of fertilizers applied to the various plots are indicated in Table 1. From 1909 to 1914, each fertilizer treatment was applied to the entire plot. In the latter year, however, all plots were divided in half. Since 1914, with the exception of farm manure, the quantity of fertilizer that was formerly applied to the entire plot has been applied to the south half of each plot, thereby doubling the former rate of application on this part of each plot, except the manure plots. From 1917 to the present time, the rates of application per acre have been as follows: Dried blood, 308 pounds; ammonium sulphate, 200 pounds; rock phosphate, 219 pounds; superphosphate, 438 pounds; muriate of potash, 200 pounds; and manure,

[1] Received for publication Dec. 28, 1931; issued July, 1932.

[2] The writer is indebted to the Departments of Agricultural Chemistry and of Botany and Plant Pathology for laboratory facilities in making the chemical analyses reported in this paper.

[3] Reference is made by number (italic) to Literature Cited, p. 930.

16 tons once in four years, except plot 12, which received 4 tons annually. All applications were made annually except as noted otherwise.

The sap samples were obtained from 15-inch sections of the stalks immediately above the surface of the soil. These sections were cut into small pieces approximately an inch in length, and subjected to a pressure of 6,500 pounds per square inch in a small Carver laboratory hydraulic press. The sap samples thus obtained in 1928 and 1929 were clarified immediately with carbon black and then stored in a refrigerator at −7° C. until removed for analysis. The samples obtained in 1930 and 1931 were not clarified. A small amount of toluene was added to each sample to prevent fermentation.

The samples were collected during the last week in August each year. In 1928, a second set of samples was taken during the last week in September.

Chlorine content was determined as follows: 10 c c of sap were placed in an evaporating dish with 20 c c of a 5 per cent sodium-carbonate solution, evaporated to dryness, ashed at a very low temperature, taken up with water, and filtered. The filtrate was then titrated with a standard silver nitrate solution in the presence of a yellow light, potassium chromate being used as indicator. The titrations were made under a yellow light because the potassium-chromate indicator inparts a yellow color to the solution to be titrated and, hence, in order to sensitize the end point properly, it was desirable to have the solution and the light of the same or similar color and shade of color. A yellow 25-watt incandescent lamp was used as the source of light. When the chlorine analyses were first undertaken, considerable difficulty was experienced in getting the samples to ash completely at temperatures sufficiently low to prevent volatilization of the chlorides. This difficulty was overcome by moistening the incompletely ashed residue with water, redrying in an electric oven, and then returning the sample to the furnace. In some cases a second moistening was necessary for complete combustion.

PRESENTATION, INTERPRETATION, AND DISCUSSION OF EXPERIMENTAL RESULTS

EFFECT OF FERTILIZERS CONTAINING CHLORINE ON THE CHLORINE CONTENT OF THE PLANT SAP

The results obtained in this investigation show a number of interesting relations. (Table 1 and fig. 1.) The most evident of these is the marked effect that chlorine-carrying fertilizers had on the chlorine content of the plant sap. Considering for the moment only the 4-year averages for the samples collected in August, it will be found that the plots which have received no chlorine at any time during the experiment (except in crop residues) yielded saps containing less than 0.20 mg of chlorine per cubic centimeter (200 parts per million). On the other hand, saps coming from plots which received muriate of potash annually, contained more than 1.60 mg of chlorine per cubic centimeter (1,600 p. p. m.), an increase of over 700 per cent. The manure plots yielded saps which contained between 0.50 and 1.10 mg of chlorine per cubic centimeter (500 to 1,100 p. p. m.). They were therefore distinctly intermediate between the saps from untreated plots and those from plots which received muriate of potash annually.

In most cases, the data from individual years do not deviate greatly from the averages just considered. It is evident from these data, therefore, that the application of chlorine-carrying fertilizers increased greatly the chlorine content of the sap of corn plants.

TABLE 1.—*Chlorine content of sap extracted from corn plants grown on plots receiving different fertilizer treatments*

[Values are in milligrams of chlorine per cubic centimeter of sap]

Plot No.	Treatment [a]	Chlorine in sap extracted on—					Average August samples	Average all samples
		Aug. 29, 1928	Sept. 28, 1928	Aug. 30, 1929	Aug. 30, 1930	Aug. 27, 1931		
		Mg	*Mg*	*Mg*	*Mg*	*Mg*	*Mg*	*Mg*
$2D_1$ [b]	O		0.14	0.14	0.18	0.07	0.13	0.13
$2D_2$	Ps	0.12	.09	.15	.16	.17	.15	.14
$3D_1$	O	.34	.26	.15	.19	.18	.22	.22
$3D_2$	N, Ps, K	3.21	2.86	1.32	1.29	1.44	1.82	2.02
$4D_1$	Check	.19	.40	.18	.18	.12	.17	.21
$4D_2$	N(ams), Pr	.20	.13	.16	.11	.06	.13	.13
$5D_1$	O	.17	.36	.19	.26	.46	.27	.29
$5D_2$	Ps, K	2.82	2.54	2.39	.96	2.26	2.11	2.19
$6D_1$	O	.47	.85	.35	.26	.45	.38	.48
$6D_2$	K	2.59	2.13	1.31	1.06	1.74	1.68	1.77
$7D_1$	O	.21	.78	.35	.17	.25	.25	.35
$7D_2$	N, K	2.48	2.89	1.26	1.22	1.11	1.52	1.79
$8D_1$	O	.04	.37	.13	.12	.08	.09	.15
$8D_2$	N, Ps	.16	.31	.18	.15	.11	.15	.18
$9D_1$	O	.19	.29	.17	.15	.08	.15	.18
$9D_2$	N	.13	.15	.17	.14	.12	.14	.14
$10D_1$	O	.11	.15	.12	.11	.10	.11	.12
$10D_2$	M	2.03	2.26	.66	.50	.70	.97	1.23
$11D_1$	Check	.13	.29	.12	.10	.17	.13	.16
$11D_2$	M, Pr	2.57	2.98	.78	.60	.58	1.13	1.50
$12D_1$	O	.17	.35	.09	.09	.10	.11	.16
$12D_2$	M	.76	1.13	.36	.58	.51	.55	.67
$13D_1$	O	.20	.11	.09	.12	.07	.12	.12
$13D_2$	M, Ps		2.42	.59	.95	.61	.72	1.14
$14D_1$	O		.36	.18	.18	.16	.17	.22
$14D_2$	Pr	.14	.18	.05	.16	.13	.12	.13

[a] Check, no treatment since 1909; O, no treatment since 1914; N, nitrogen from dried blood; N(ams), nitrogen from ammonium sulphate; M, manure; Pr, rock phosphate; Ps, superphosphate; K, muriate of potash.

[b] All plots on range D in 1928, range C in 1929, range B in 1930, and range A in 1931.

The failure of the saps from the manure plots to contain as much chlorine as those from plots which received muriate of potash raises the question whether these differences in chlorine content might possibly be related to differences in the amounts of chlorine supplied by the two fertilizers. The manure which has been used in the past on these plots has been produced by work horses kept at the experiment station. These animals are usually bedded with cereal straws and fed on various grass and legume hays. Van Slyke (*19, p. 83*) reports the chlorine content of oat straw as being 0.31 per cent. This figure is presumably for air-dry straw, and it would be logical to expect fresh manure to show a much lower chlorine content when analyzed. This was confirmed by an analysis of four samples collected individually and at random from the manure now in storage at the experiment station barns, which showed chlorine contents of 0.13, 0.15, 0.18, and 0.18 per cent, respectively, on the wet basis. The moisture content of the four samples was 72.4, 67.6, 67.5, and 68.3 per cent, respectively. On the basis of these analyses a ton of manure contains approximately 3.2 pounds of chlorine. Since the manure was applied at the rate of 16 tons per acre in four years, the plots received approximately 51

pounds of chlorine for each rotation. Muriate of potash applied annually at the rate of 200 pounds per acre supplied approximately 375 pounds of chlorine in each 4-year period, nearly eight times as much as the manure furnished. It is clear from these figures that

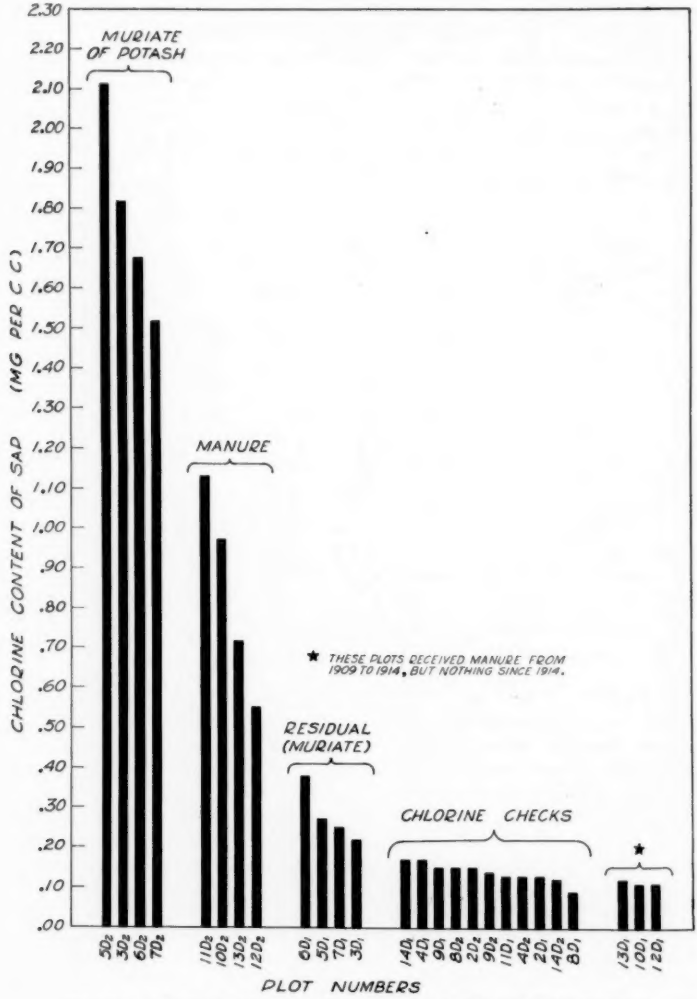

FIGURE 1.—Chlorine content of sap of corn plants as affected by fertilizer treatment

the lower chlorine content of the saps from the manure plots was due to the smaller amount of chlorine supplied by the manure, and that the higher concentration in the sap from the muriate of potash plots was caused by the larger amount of chlorine supplied by the muriate.

It is also clear that although muriate of potash increased the chlorine content of the plant sap more than manure did, the increase is not proportional to the amount of chlorine applied. The saps from the muriate of potash plots contained about twice as much chlorine as those from the manure plots, but this increase is far from being directly proportional to the 375 pounds of chlorine supplied by the muriate per rotation as compared to 51 pounds supplied by manure. It is not possible to account for this difference on the basis of differential rates of utilization of chlorine within the plants, because this element enters into practically no organic compounds occurring naturally in plants except the anthocyanin pigments. Hence, the amount of chlorine assimilated by corn plants must be very small, and differential utilization would bring about only small differences. It is possible, however, that the large amount of chlorine supplied by the muriate may have exceeded the capacity of the corn plant to absorb and retain this element. In this event, applications of chlorine larger than the amount necessary to extend the plant to its capacity in this respect, would effect no increase in the chlorine concentration of the sap.

EFFECT OF DATE OF SAMPLING ON THE CHLORINE CONTENT OF THE PLANT SAP

As previously pointed out, two sets of samples were collected in 1928, the first during the last week in August and the second during the last week in September. The data in Table 1 show that in 16 of the 23 comparisons the September samples contained more chlorine than the samples obtained from the same plots a month earlier. This indicates that in many of the plots chlorides continued to move into the plants until they were nearly mature. Similar results have been reported by Harris (*3*), who found that chlorides accumulated in the leaf-tissue fluids of cotton plants with the march of the season.

It is of interest also to compare the September movement of chlorides in the various plots with their respective fertilizer treatments. Where no chlorine has been applied since 1914, 75 per cent of the total number of saps show a higher chlorine content in September than in August. Where muriate of potash has been applied, however, 75 per cent of the total number of saps show a lower chlorine content in September. The saps from the manure plots show higher chlorine concentrations in the September samples.

The increases in concentration of chlorine in the saps from the untreated and manure plots and the decreases in the saps from the plots which received muriate of potash are difficult to reconcile. In the case of increases, it is natural to assume that the plants continued to absorb chloride ions during September in such quantities as to accumulate an observable increase. It is possible, however, that a part of the increases in chlorine concentration was caused by (1) a loss of water during maturation, or (2) by the movement of chlorine into the stalk from other parts of the plant. Desiccation incident to maturation is not believed to have had much influence, for there appeared to be as much or more sap in the stalks in September than in August. Furthermore, no appreciable differences were observed in the time of maturity of the corn on the various plots. The movement of chlorine into the stalks from other parts of the plant, par-

ticularly from the maturing leaves, remains as a possible partial explanation, for it is well known that when plants ripen the salts held in the sap have a tendency to migrate from the dying to the living tissues. This possibility is strengthened by the fact that approximately nine-tenths of the chlorine in cereal plants is contained in the stem tissues (*19, p. 83*).

The lower chlorine concentrations in the September samples where muriate of potash was applied may have resulted from one or more of three different causes. First may be mentioned the much disputed theory of the outward movement of materials from the plant back to the soil through the roots. For information concerning this theory, the reader is referred to a recent critical review by Thomas (*17*). Secondly, it is known that chlorine-containing compounds in plants occur not only dissolved in the cell sap, but also as deposits of definite crystals or amorphous compounds in the cell protoplasm (*16, p. 115*). If conditions within the plant during maturation become such as to increase the crystallization and precipitation of chlorine compounds and thus prevent their removal with the sap, the observed decreases in chlorine concentration of the September samples could be accounted for. In the third place, it has been shown by Le Clerc and Breazeale (*12*) that from 40 to 75 per cent of the chlorine in various crop plants may be washed out by rains and returned to the soil. They point out further that this occurs to a greater extent during maturation than before. Since the rainfall on these plots during September was 6.62 inches, a part of the chlorine losses may have occurred in this way.

Garner and his associates (*1*) found that a high chlorine content in the leaf of tobacco was accompanied by an increase in its water content. James (*11*) has recently observed similar increases in the water content of the leaves of potato plants where either muriate of potash or potash manure salts had been applied. If considered casually, these observations would seem to explain the lower chlorine concentrations observed in the studies reported here by assuming that the concentrations were lowered through an increased absorption of water instead of a loss of chlorine. A close examination of Garner's data, however, shows that where muriate of potash was applied the increases in water content are much smaller than the increases in chlorine content. James has reported no chlorine analyses, and hence it is not possible to determine the status of this relation in his studies. On the basis of Garner's data, the hygroscopicity of chlorides can not explain the lower chlorine concentrations in the September samples where muriate of potash was applied, unless it is assumed that (1) the capacity of the plants for absorbing chlorides had been completely satisfied during both August and September, and (2) that the increase in water content did not develop until September. Some indirect evidence has already been presented to support the first assumption, but the latter is unsupported and probably was not operative in the studies reported here.

RESIDUAL CHLORINE

It is interesting to note from Figure 1 that although the saps from the untreated ends of plots 3, 5, 6, and 7 contained less chlorine than the saps from the manure and muriate of potash plots, they contained

more chlorine than the saps from check plots. As previously stated, these four plots received 100 pounds of muriate of potash per acre annually from 1909 to 1914. In 1914 they were divided into halves and the fertilized halves have since received the same amounts of fertilizer as previously applied to the entire plot, making the rate of application for muriate of potash 200 pounds per acre since 1914. The unfertilized halves have received no fertilizer since 1914. In view of the marked effect that muriate of potash exerted on the chlorine content of the plant sap when applied annually from 1909 to 1931 inclusive (fig. 1), it is evident that the saps from the untreated ends of plots 3, 5, 6, and 7 show the residual effects of the muriate of potash applied from 1909 to 1914. The saps from these plots also show the residual effects of the potassium applied in the muriate of potash (*14, Table 2*).

The saps from the untreated ends of plots 10, 12, and 13, which received farm manure from 1909 to 1914 but nothing since the latter date, show no residual effects of the chlorine supplied by the manure approximately 15 years ago. In fact, the saps from these plots contained slightly less chlorine than most of the check plots. These data, therefore, seem to be at variance with the generally accepted belief that organic fertilizers, especially farm manures, have residual effects of longer duration than do inorganic fertilizers (*13, p. 614; 20, p. 221*). It must be kept in mind, however, that the manure supplied less than one-third as much chlorine as the muriate of potash supplied. Furthermore, the manure plots have consistently returned the highest crop yields, and therefore have manifested a larger demand for chlorine than the other plots. Hence, the larger demand for chlorine occurred on the plots to which the smaller amounts were formerly applied. Over the intervening period of about 15 years this appears to have been sufficient to exhaust the chlorine which remained as residual material when the manure treatment was discontinued on these plots.

The annual yield data from the plots which formerly received muriate of potash show a strong tendency for the residual chlorine to be inversely proportional to crop growth. (Fig. 2.) The yielding power of these plots has thus far been determined largely by the degree of fertilization, i. e., whether a complete fertilizer was applied or one carrying but one or two of the three major fertilizer constituents, nitrogen, phosphoric acid, and potash. In general, the plots which have received but one constituent have given the lowest yields; those which have received two constituents have returned fair yields, while the plots which have received a complete fertilizer have given the highest yields. Of the plots showing residual chlorine, plot 3, which received a complete fertilizer from 1909 to 1914, shows the least residual effect. This plot has a crop yield index of 142 since 1914. Plot 6, on the other hand, which formerly received muriate of potash only, has a yield index of only 104 and shows the strongest residual effect. Plots 5 and 7, each of which formerly received fertilizers carrying two of the three major constituents, show intermediate residual effects. The yield index of 125 for plot 5 is also intermediate, but the index of 101 for plot 7 is low. On the whole, these data show that the more complete fertilization previous to 1914 has caused greater crop growth since fertilization was discontinued, which, in turn, resulted in

a heavier withdrawal of the soil's supply of chlorine and therefore a weaker residual effect. Poor fertilization caused inferior crop growth, less absorption of chlorides, and therefore a stronger residual effect.

THE CHLORINE : POTASH RATIO

Garner and his associates (*1*) have recently reported that where muriate of potash is used on tobacco, the plant contains approximately as much chlorine as potash. They attach considerable significance to the chlorine: potash ratio because the potassium occurring in the form of chloride is not available for the formation of salts of organic acids, except as replaced by other metals. This is apparently important in tobacco because a high chlorine content as well as a low content of potash salts of organic acids both have an unfavorable effect on the burning quality of the tobacco leaf.

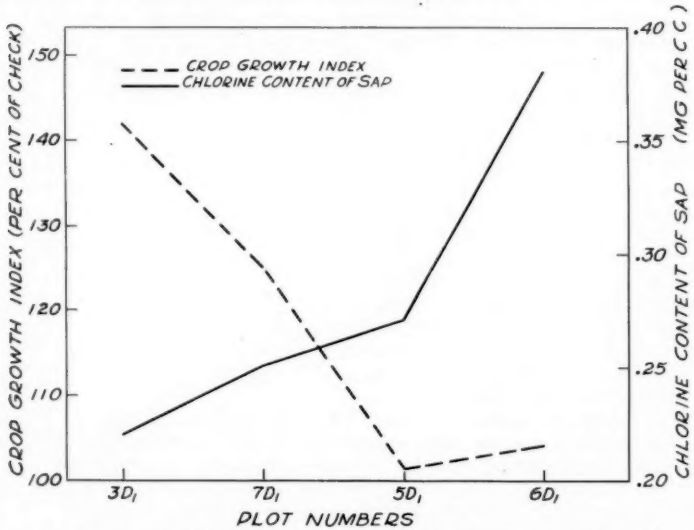

FIGURE 2.—Inverse relationship between crop yields and chlorine content of sap of corn plants grown on plots showing residual effects of chlorine applied approximately 15 years ago

Although the physiology of the corn plant is probably very different from that of the tobacco plant in relation to the development of qualities that are of importance in the crop, it may be of interest to compare the amounts of potash and chlorine occurring in the sap of corn plants and to determine the effect of various fertilizers on the chlorine: potash ratio. Accordingly, the ratios have been calculated and are given in Table 2. It is immediately clear from the data that there is much less chlorine than potash in the sap of corn plants. Nearly all of the 1929, 1930, and 1931 ratios lie between 0.1:1.0 and 0.3:1.0, which means that in these three years there was from 10 to 30 per cent as much chlorine in the sap as there was potash. The ratios for 1928 are comparatively high for some reason, many being as high as 0.4 and 0.5.

TABLE 2.—*Ratio of chlorine to potash in the sap of corn plants grown on plots receiving different fertilizer treatments*

Plot No.	Treatment[a]	Ratio of chlorine to potash when potash equals 1			
		Aug. 29, 1928	Aug. 30, 1929	Aug. 30, 1930	Aug. 27, 1931
$2D_1$[b]	O		0.06	0.16	0.15
$2D_2$	Ps	0.21	.13	.15	.28
$3D_1$	O	.48	.07	.23	.20
$3D_2$	N, Ps, K	.54	.33	.26	.23
$4D_1$	Check	.41	.19	.10	.11
$4D_2$	N(ams), Pr	.56	.21	.20	.12
$5D_1$	O	.22	.14	.25	.32
$5D_2$	Ps, K	.47	.33	.19	.38
$6D_1$	O	.21	.22	.27	.23
$6D_2$	K	.48	.24	.28	.38
$7D_1$	O	.40	.15	.23	.24
$7D_2$	N, K	.44	.26	.26	.21
$8D_1$	O	.50	.11	.21	.13
$8D_2$	N, Ps	.36	.22	.23	.12
$9D_1$	O	.31	.30	.21	.27
$9D_2$	N	.33	.31	.21	.43
$10D_1$	O	.21	.22	.14	.17
$10D_2$	M	.33	.14	.10	.16
$11D_1$	Check	.29	.34	.14	.17
$11D_2$	M, Pr	.40	.14	.10	.16
$12D_1$	O	.19	.18	.16	.09
$12D_2$	M	.12	.06	.09	.10
$13D_1$	O	.28	.16	.17	.07
$13D_2$	M, Ps		.12	.19	.13
$14D_1$	O		.16	.14	.11
$14D_2$	Pr	.27	.07	.04	.15

[a] Check, no treatment since 1909; O, no treatment since 1914; N, nitrogen from dried blood; N(ams), nitrogen from ammonium sulphate; M, manure; Pr, rock phosphate; Ps, superphosphate; and K, muriate of potash.

[b] All plots on range D in 1928, range C in 1929, range B in 1930, and range A in 1931.

Concerning the effect of fertilizer treatment on the proportion of chlorine to potash in the sap, the ratios are not entirely consistent. In general, the effect of manure was to lower the chlorine:potash ratio. If sap composition is assumed to be an index of the supply of available nutrients in the soil (*14*), this indicates that manure supplied the plants with more potash than chlorine. Muriate of potash, on the other hand, usually increased the proportion of chlorine to potash. The increases are relatively small, however, in view of the fact that chlorine and potassium were added in approximately equal amounts.

Attention has already been called to the fact that Garner and his associates (*1*) found as much chlorine as potash in the stalks and leaves of tobacco plants where muriate of potash was used in the fertilizer. Where no muriate was used, the proportion of chlorine to potash was similar to the ratios given in Table 2. Since the chlorine:potash ratios reported here for corn sap do not even remotely approach the proportions of 1:1 where muriate of potash was used, it would seem that as compared to tobacco the corn plant has a very limited capacity for absorbing and retaining the chloride ion. It must be kept in mind, of course, that Garner's data were obtained from analyses of dehydrated stalk and leaf tissues, and that the ratios reported here represent only the sap fraction that is extractable at 6,500 pounds of pressure per square inch. However, the quantities of potassium and chlorine present in the expressed sap probably give a fair estimate of their relative abundance in the plant,

because it is well known that although these elements enter into organic combinations to some extent in the plant, they generally remain dissolved in the cell sap and are therefore subject to extraction with this part of the plant. These differential capacities of corn and tobacco for absorbing chlorides from the soil solution are undoubtedly characteristic of the two species of plants and inherent in them. Harris and his associates (*5*, *6*, *8*) found that Egyptian cottons have a larger capacity for absorbing chlorides than do upland cottons. Upon crossing these two types of cotton, they found this character to be heritable; the F_1 progeny were intermediate and the F_2 progeny showed segregation for the ability to absorb selectively either chlorides or sulphates.

RELATIVE ABSORPTION OF CHLORIDE AND SULPHATE IONS

A number of investigators have shown that plants absorb the chloride ion much more readily than the sulphate ion. Reference to such work will be limited here to the controlled culture work of Hoagland (*9*) and Hoagland and Martin (*10*) at the California station and to the extensive field experiments of Garner and his associates (*1*) in the United States Department of Agriculture. The results obtained by these men, as well as those of many others, show that the greater absorption of the chloride ion is not limited to a few species of plants, but is quite common throughout the plant kingdom. The only exception that has come to the writer's attention is the larger sulphate content found by Harris and his associates (*4*, *5*, *8*) in the leaf-tissue fluids of cotton plants grown on alkali soils in Arizona.

In order to learn whether the general experience of other workers concerning the relative absorption of chloride and sulphate ions was true of the corn plant, an attempt was made to determine the sulphate content of the sap samples gravimetrically. It was soon found, however, that the saps contained such small amounts of sulphates that it would be impossible to make quantitative determinations unless large samples were used. Even the saps from the treated end of plot 4, where 200 pounds of sulphate of ammonia have been applied annually since 1914, were practically devoid of sulphates. Previous analyses for other materials consumed nearly all the sap available, and since neither nephelometric nor micro equipment suitable for determining small amounts of sulphate was available, it was impossible to make quantitative estimations of the sulphate content of the various sap samples. It is clear from this experience and from the fact that 10 c c of sap was sufficient to ascertain the chlorine content of the samples, that the chloride ion is much more abundant in the sap of corn plants than is the sulphate ion. It is well known that some of the sulphur contained in sulphates is transformed into certain proteins and glucosides within the plant, but it is very improbable that such utilization would account for the large differences observed in the chloride and sulphate content of the saps from the various plots. The relative ease with which muriate of potash and farm manure increased the chlorine content of the sap and the failure of sulphate of ammonia to increase the sulphate content appreciably, makes it quite clear that corn plants absorb the chloride ion much more readily than the sulphate.

This differential absorption of chlorides and sulphates is undoubtedly dependent on (1) the structure, chemical composition, and pore spaces of the root-absorbing surfaces, and (2) on the degree of hydration, ionic volume, and degree of dissociation of salts (*18*). Concerning the latter, it should be noted that the physical and chemical properties of the two ions are distinctly different. Thus, it is well known that the chlorides are not only more soluble than the sulphates, but also that they dissociate to a greater degree. Furthermore, the sulphate ion is approximately three times heavier and larger than the chloride ion. Chloride ions therefore have a greater diffusion velocity than sulphate ions, and, being smaller in volume, have less difficulty in passing through the pore spaces of the root-absorbing membranes. These differences between the two ions are magnified still more by their differential degrees of hydration, for most sulphates attract and hold more molecules of water than chlorides do. This increases the weight and volume and decreases the diffusion velocity of the sulphate ions to a greater extent than for the chloride ions, and imposes a greater handicap on the former than on the latter in passing through the root membranes. It is clear, then, that such influences as solubility, degree of dissociation, ionic volume, and degree of hydration, are all more favorable to the absorption of chloride ions than sulphate ions. Nevertheless, it is unlikely that these differences in the chemical and physical properties of the two ions alone can account for their differential absorption by plants, for Stiles (*15*) was unable to show any correlation between the rates of diffusion of sulphate and chloride ions into gels and into living cells. It is now generally recognized that the structure and chemical composition of the cell membranes must be taken into consideration as well as the physical and chemical properties of the ions.

The rate of absorption of chlorides and sulphates from soils of pH 5.5 to 7 corresponds to their relative positions in the Hofmeister series for anions, which, in descending order of mobility, is $OH > I$, $Br > NO_3 > Cl > HPO_4$, SO_4 (*2*, *18*).

HYDROGEN-ION CONCENTRATION OF SAP AS AFFECTED BY CHLORINE CONTENT

In a previous report (*14*, *p. 108*) on the corn-sap studies now under way at the Virginia station, attention was called to a fairly close relationship between the acidity of the expressed sap and potassium fertilization. In general, the saps from plots where muriate of potash had been applied were most acid (below pH 5.40), those from the manure plots were somewhat less acid, while those from plots where no potash materials had been added were least acid. If these observations are correlated with the chlorine concentrations in the sap (Table 1), it will be found that there is a positive relation between (1) the amount of chlorine supplied by the fertilizer, (2) the chlorine content of the expressed plant sap, and (3) the hydrogen-ion concentration of the sap. The saps from the plots which received muriate of potash were more acid than the others because the large amount of chlorine supplied by the fertilizer caused a large accumulation of chloride ions within the plant. Manure supplied less chlorine, caused a smaller accumulation of chloride ions within the plant, and a lower degree of acidity. Where no chlorine was applied in the fertilizer, there was little accumulation of chloride ions within the

plant, and the expressed sap was still less acid. It is clear, then, that because of the relative ease with which the corn plant absorbs and accumulates chloride ions, the amount of chlorides at its disposal is one of the important factors governing the reaction of the cell sap. The soil's supply of sulphates, on the other hand, apparently plays very little part directly in determining the reaction of the sap of corn plants, because of the difficulty with which this ion is absorbed.

The observations reported here with reference to a positive correlation between hydrogen-ion concentration and chlorine content of the sap of corn plants are in accord with those of Harris and his associates (*7*, *8*), who found that the leaf-tissue fluids of Egyptian cottons have a higher chlorine content and are more acid than those of upland cottons.

SUMMARY AND CONCLUSIONS

The use of fertilizers containing chlorine increased the chlorine content of the sap of corn plants. The increase is partially proportional to the amount of chlorine supplied by the fertilizer.

Many of the sap samples contained slightly more chlorine in September than in August. Where muriate of potash had been used, there was a slight decrease in chlorine content during September.

Chlorine added in muriate of potash approximately 15 years ago is still exerting a residual effect on the chlorine content of the plant sap. There is a tendency for this residual effect to be inversely proportional to crop yields.

The chlorine supplied by manure 15 years ago is exerting no residual effect at present.

The sap of corn plants contains approximately one-fourth as much chlorine as potash. Muriate of potash increased the chlorine:potash ratio slightly, while manure lowered it slightly.

Fertilizers containing sulphur failed to cause an appreciable accumulation of sulphate ions in the sap of corn plants.

Corn plants absorb the chloride ion much more readily than the sulphate ion.

As the chlorine content of the sap increases, the hydrogen-ion concentration increases also.

LITERATURE CITED

(1) Garner, W. W., McMurtrey, J. E. Jr., Bowling, J. D., and Moss, E. G.
1930. RÔLE OF CHLORINE IN NUTRITION AND GROWTH OF THE TOBACCO PLANT AND ITS EFFECT ON THE QUALITY OF THE CURED LEAF. Jour. Agr. Research 40:627–648, illus.

(2) Hager, G., and Stollenwerk, W.
1930. IN WELCHEM MASSE KÖNNEN DIE AMMONIAKSALZE DEN BODEN DURCH DIE BEVORZUGTE AUFNAHME DES AMMONIUMKATIONS IM VERGLEICH ZUM ANION VERSAURN?—EIN BEITRAG ZUR ERKENNTNIS DER NÄHRSTOFF-AUFNAHME DURCH DIE PFLANZEN. Ztschr. Pflanzenernähr u. Düngung (A) 17:[129]–165.

(3) Harris, J. A.
1925. THE ACCUMULATION OF CHLORIDES IN THE LEAF TISSUE FLUIDS OF EGYPTIAN COTTON WITH THE MARCH OF THE SEASON. Soc. Expt. Biol. and Med. Proc. 22:415–417.

(4) ——— Hoffman, C. T., and Hoffman, W. F.
1925. SULPHATE CONTENT OF THE LEAF-TISSUE FLUIDS OF EGYPTIAN AND UPLAND COTTON. Jour. Agr. Research 31:653–661.

(5) HARRIS, J. A., HOFFMAN, W. F., and LAWRENCE, J. V.
1925. DIFFERENTIAL ABSORPTION OF ANIONS BY VARIETIES OF COTTON. Soc. Expt. Biol. and Med. Proc. 22:350–352.
(6) ——— HOFFMAN, W. F., SINCLAIR, W. B., JOHNSON, A. H., and EVANS, R. D.
1925. THE LEAF-TISSUE FLUIDS OF EGYPTIAN COTTONS. Jour. Agr. Research 31:1027–1033.
(7) ——— LAWRENCE, Z. W., HOFFMAN, W. F., LAWRENCE, J. V., and VALENTINE, A. T.
1924. THE TISSUE FLUIDS OF EGYPTIAN AND UPLAND COTTONS AND THEIR F_1 HYBRID. Jour. Agr. Research 27:267–328, illus.
(8) ——— LAWRENCE, J. V., and LAWRENCE, Z. W.
1924. THE CHLORID CONTENT OF THE LEAF TISSUE FLUIDS OF EGYPTIAN AND UPLAND COTTON. Jour. Agr. Research 28:695–704.
(9) HOAGLAND, D. R.
1923. THE ABSORPTION OF IONS BY PLANTS. Soil Sci. 16:225–246, illus.
(10) ——— and MARTIN, J. C.
1923. EFFECT OF SALTS ON THE INTAKE OF INORGANIC ELEMENTS AND ON THE BUFFER SYSTEM OF THE PLANT. Calif. Agr. Expt. Sta. Tech. Paper 8, 26 p., illus.
(11) JAMES, W. O.
1930. STUDIES OF THE PHYSIOLOGICAL IMPORTANCE OF THE MINERAL ELEMENTS IN PLANTS. I. THE RELATION OF POTASSIUM TO THE PROPERTIES AND FUNCTIONS OF THE LEAF. Ann. Bot. [London] 44:[173]–198, illus.
(12) LE CLERC, J. A., and BREAZEALE, J. F.
1909. PLANT FOOD REMOVED FROM GROWING PLANTS BY RAIN OR DEW. U. S. Dept. Agr. Yearbook 1908:389–402.
(13) LYON, T. L., FIPPIN, E. O., and BUCKMAN, H. O.
1917. SOILS, THEIR PROPERTIES AND MANAGEMENT. 764 p., illus. New York.
(14) PETTINGER, N. A.
1931. THE EXPRESSED SAP OF CORN PLANTS AS AN INDICATOR OF NUTRIENT NEEDS. Jour. Agr. Research 43:95–119, illus.
(15) STILES, W.
1921. THE PENETRATION OF ELECTROLYTES INTO GELS. IV. THE DIFFUSION OF SULPHATES IN GELS. Biochem. Jour. 15:629–635.
(16) THATCHER, R. W.
1921. THE CHEMISTRY OF PLANT LIFE. 268 p. New York.
(17) THOMAS, W.
1930. THE FEEDING POWER OF PLANTS. Plant Physiol. 5:443–489.
(18) ———
1932. THE RECIPROCAL EFFECTS OF NITROGEN, PHOSPHORUS, AND POTASSIUM AS RELATED TO THE ABSORPTION OF THESE ELEMENTS BY PLANTS. Soil Sci. 33:1–20, illus.
(19) VAN SLYKE, L. L.
1927. FERTILIZERS AND CROPS; OR, THE SCIENCE AND PRACTICE OF PLANT-FEEDING; A PRESENTATION OF FACTS, GIVING PRACTICAL METHODS FOR USING FERTILIZERS IN CROP GROWING, WITH SPECIAL EMPHASIS ON THE REASONS UNDERLYING THEIR USE, AND ON THE CONDITIONS OF THEIR GREATEST EFFICIENCY. 734 p., illus. New York and London.
(20) WEIR, W. W.
1920. PRODUCTIVE SOILS; THE FUNDAMENTALS OF SUCCESSFUL SOIL MANAGEMENT AND PROFITABLE CROP PRODUCTION. 398 p., illus. Philadelphia and London.

U. S. GOVERNMENT PRINTING OFFICE: 1932

INDEX

Page

Acetic Acid—
and Pyroligneous Acid, Comparison with Formaldehyde as Soil Disinfectants. William L. Doran ... 571–578
in soil, effect on damping off of red pine seedlings ... 575, 577–578
Age—
alfalfa seedlings, influence on cold resistance ... 434–435
dairy cattle, effect on blood-sugar content ... 359, 364
Agricultural Research, Use of Photographic Light Box. A. B. Groves ... 467–475
AIKINS, GLENN A., and FAY, A. C.—
Effect of Light on the Reduction of Methylene Blue in Milk ... 85–95
Factors Influencing the Changes in Oxidation-Reduction Potential on the Reduction of Methylene Blue in Milk ... 71–83
Aleurone color, in corn, genetical study ... 495–498, 502
Alfalfa seedlings—
age, influence on cold resistance ... 434–435
freezing, experiments ... 431–435, 438–441, 444
handling after freezing, methods ... 441–442
hardening, experiments ... 435–439
planting and growing, experiments ... 429–431
survival after freezing, factors affecting ... 431–434
Alfalfas—
cold resistance, tests ... 442–444
Seedling, Comparative Hardiness in, Method for Determination by Controlled Hardening and Artificial Freezing. George L. Peltier and H. M. Tysdal ... 429–444
Algae, effect on mosquito breeding rates and plankton content of water ... 391–393
ALICATA, JOSEPH E.: Life History of the Rabbit Stomach Worm, *Obeliscoides cuniculi* ... 401–419
ALLARD, H. A.: Flowering Behavior of the Hog Peanut in Response to Length of Day ... 127–137
ALLEN, RUTH F.: A Cytological Study of Heterothallism in *Puccinia triticina* ... 733–754
Alternaria radicina, growth on carrots in storage ... 896, 902–905
Ammonia nitrogen, content of decomposing green manure ... 114, 116, 118, 120
Ammonium sulphate, effect on chlorine content of corn sap, study ... 919–921
Ancylis comptana—
(Froel.), Strawberry Leaf Roller in New Jersey, Biology and Habits. David E. Fink ... 541–558
synonymy ... 543
See also Strawberry leaf roller.
Ancylostoma caninum, exsheathing in aniline stain ... 409
Andropogoneae, genera of, chromosome numbers, study ... 321
Aniline stain, effect on larvae of—
Ancylostoma caninum ... 409
Obeliscoides cuniculi ... 408–409, 418
Anopheles—
breeding, factors affecting ... 388, 391–396
larvae—
abundance, influence of temperature on ... 392–388
adaptability to breeding places ... 390–391
dissection, studies and results ... 395–396, 399
food, study ... 396–398, 399
protection, various factors ... 388–391
larval rates, comparison with plankton content of water ... 386–387
Mosquitoes, Breeding, Associated Factors. G. H. Bradley ... 381–399

Page

Anthesis, Tomato, and Blossom Drop, Relation to Temperature, together with a Histological Study of the Pistils. Ora Smith ... 183–190
Anthocyanin Pigment in Maize, Factors Affecting, Additional Pair. Merle T. Jenkins ... 495–502
ANTHONY, R. D., and CLARKE, W. S., JR.: Growth Record of Fertilized Apple Trees Grown in Metal Cylinders ... 245–266
Apparatus, photographic, for uniform light, description and operation ... 467–470
Apple—
and Pear, Elsinoe on. Anna E. Jenkins ... 689–700
leaves, injury by oil spray ... 777
Trees—
Fertilized, Growing in Metal Cylinders, Growth Record. R. D. Anthony and W. S. Clarke, Jr ... 245–266
growth and yield records, correlations ... 263–265
leaf weight and branch elongation, record ... 252–257
planting and care, in fertilizer study ... 246–248
tops and roots, weight, measurement ... 258–261
trunk diameter, increase, measurement ... 257–258
Apples, weight, measurement ... 262–263
Ash canker—
disease—
damage to trees ... 702, 708
treatment, recommendations ... 721–722
organism, tests and comparisons with olive-tubercle organism ... 708–721, 722
Ash Trees—
Canker of, Production by Variety of Olive-Tubercle Organism, *Bacterium sexatanei*. Nellie A. Brown ... 701–722
inoculation with *Bacterium sexatanei*, experiments ... 704–708

Bacillus—
carotovorus, growth on carrots in storage, study ... 861, 863, 871, 879–886, 890, 891, 896–897, 900–905, 909–910
sp., cause of spoilage of dressed ducklings, study ... 914, 918
Bacteria—
phytopathogenic, disintegration of cell walls ... 625–631, 632
soil, number in decomposing green manure, study ... 113, 116, 118–119, 120
Bacterial—
blight of beans—
causal organisms, study ... 605–632
symptoms caused by three bacteria ... 605–607
Disease of Bean, Three, Comparative Pathological Histology. W. J. Zaumeyer ... 605–632
soft rot, of carrots, development, experiments ... 861–863, 879–886, 896–906, 909–910, 911
Bacterium—
flaccumfaciens—
comparison with *Bact. medicaginis phaseolicola* and *Bact. phaseoli* ... 605–632
relation to host tissues ... 618–625, 631–632
medicaginis phaseolicola—
comparison with *Bact. flaccumfaciens* and *Bact. phaseoli* ... 605–632
relation to host tissues ... 618–618, 631–632
phaseoli—
comparison with *Bact. flaccumfaciens* and *Bact. medicaginis phaseolicola* ... 605–632
relation to host tissues ... 606–613, 631–632
sexatanei—
comparison with *Bacterium sexatanei fraxini*, n. var ... 721, 722

Page

Bacterium—Continued.
sesostanoi—Continued.
frazini, n. var., comparison with *Bacterium sesostanoi* ... 721, 722
Variety of Olive-Tubercle Organism, Cause of Canker of Ash Trees. Nellie A. Brown ... 701-722
Barley—
botanical characters, interrelationship ... 458-466
Catalase Activity, Relation to Growth Curve. Merritt N. Pope ... 343-355
chlorophyll deficiencies—
interrelationship ... 451-455
relation to other botanical characters ... 455-458
Growth Curve. Merritt N. Pope ... 323-341
Growth Curve, Relation to Catalase Activity. Merritt N. Pope ... 343-355
Inheritance in. D. W. Robertson, G. W. Deming, and Dwight Koonce ... 445-466
Mendelian characters, inheritance ... 448-451, 465-466
phosphoric acid content, effect of fertilizer treatments ... 581-582, 583, 586
plant—
developmental stages ... 327-329, 332-336
growth measurement, method and reliability of data ... 323-326
length, growth studies ... 329-331, 338, 339
weight, growth studies ... 332-336, 338, 339
seedlings, injury by oil spray, experiments ... 778-780, 783-786
Bean—
Bacterial Diseases, Three, Comparative Pathological Histology. W. J. Zaumeyer ... 605-632
Growth and Susceptibility to Dry Root Rot, Relation to Hydrogen-Ion Concentration of Soil. Walter H. Burkholder ... 175-181
leaves, injury by oil spray, experiments ... 776, 778, 779
plants, invasion by bacteria, comparative study ... 608-625
seed, bacterial blight, symptoms ... 605
seedlings, bacterial blight, symptoms ... 606-607
Susceptibility to Dry Root Rot, and Growth, Relation to Hydrogen-Ion Concentration of Soil. Walter H. Burkholder ... 175-181
Beans—
bacterial blight, causal organisms, study ... 605-632
Lima—
Baby, Thresher Injury. H. A. Borthwick ... 503-510
hand-shelled, seedling injuries ... 508-509, 510
threshed, seedling injuries, types ... 503-507, 509-510
yields, relation to hydrogen-ion concentration of the soil ... 176-181
Beets—
infection with curly-top virus attenuated and restored ... 756-764
sugar content, determination by sampling, method ... 633-647, 658-661, 663, 664-668
Bender, R. C.; Maynard, L. A.; and McCay, C. M.: Vitamin A and Protein Content of Various Fish Meals ... 591-603
Black rot, development on carrots in storage ... 896, 901-902, 911
Black scorch of date palm—
pathogene, description ... 163-164, 165
pathological histology and control ... 164-165
symptoms ... 156-161
Thielaviopsis paradoxa as Cause. L. J. Klotz and H. S. Fawcett ... 155-166
Blackberry, damage by strawberry leaf roller ... 542, 557
Blair, A. W., and Prince, A. L.: The Influence of Phosphates on the Phosphoric Acid Content of the Plant ... 579-590
Blanching, peanuts, effect on antineuritic vitamin ... 853-854, 859
Blood, dried, effect on chlorine content of corn sap, experiments ... 919-921
Blood-Sugar Level, Dairy Cattle, Factors Influencing. R. E. Hodgson, W. H. Riddell, and J. S. Hughes ... 357-365

Page

Blossom Drop, Tomato, and Anthesis, Relation to Temperature, together with a Histological Study of the Pistils. Ora Smith ... 183-190
Borthwick, H. A.: Thresher Injury in Baby Lima Beans ... 503-510
Botrytis—
cinerea, growth on carrots in storage, experiments ... 861, 863, 886-900, 902-905, 910
rot, development on carrots ... 861-863, 886-906, 907, 910-911
Bradley, G. H.: Some Factors Associated with the Breeding of Anopheles Mosquitoes ... 381-399
Breed, dairy cattle, effect on blood-sugar content ... 359, 364
Breeding—
Anopheles Mosquitoes, Associated Factors. G. H. Bradley ... 381-399
broomcorn, for height inheritance ... 13-20
corn for—
glossiness, study ... 167-173
study of pigmentation ... 495-502
cotton, use of correlation coefficients ... 526-537
tobacco, for inheritance of White Burley character ... 479-493
wheat, for resistance to bunt ... 121-126
Briggs, Fred N.: Inheritance of Resistance to Bunt, *Tilletia tritici*, in Crosses of White Federation with Turkey Wheats ... 121-126
Brinsmade, J. C., Jr., and Dillman, A. C.: Dehiscence of the Boll of *Linum rigidum* and Related Species ... 21-27
Broomcorn—
crossbreeding for height inheritance ... 13-20
Height, Inheritance. John B. Sieglinger ... 13-20
Brown, Nellie A.: Canker of Ash Trees Produced by a Variety of the Olive-Tubercle Organism, *Bacterium sesostanoi* ... 701-722
Bud scorch, of date palm—
cause and symptoms ... 157
distribution ... 155
Bunt, *Tilletia tritici*, Resistance to, Inheritance in Crosses of White Federation with Turkey Wheats. Fred N. Briggs ... 121-126
Burkholder, Walter H.: Effect of Hydrogen-Ion Concentration of the Soil on the Growth of the Bean and Its Susceptibility to Dry Root Rot ... 175-181

California, San Joaquin Valley, Cotton Plants, Root Constriction. J. W. Hubbard ... 39-47
Camera, use in agricultural research ... 467-475
Canker, of Ash Trees, Production by Variety of Olive-Tubercle Organism, *Bacterium sesostanoi*. Nellie A. Brown ... 701-722
Carrot, Diseases, Storage and Transit, Development. J. I. Lauritzen ... 861-912
Carrots—
infected with Rhizopus, comparison with infected sweetpotatoes ... 876-877
shriveling, effect of humidity ... 906-907, 911
stored, losses under various conditions, study ... 862-911
varietal susceptibility to infection and decay in storage ... 869-870, 877, 886, 893, 903-906, 908-911
Casein and Yeast, Supplements to Corn and Soybean Rations, Effect on Rats and Swine. Charles L. Shrewsbury, Claude M. Vestal, and Sigfred M. Hauge ... 267-274
Catalase activity, barley—
curve ... 348-354
determination ... 344-346, 353-355
Relation to Growth Curve. Merritt N. Pope ... 343-355
Cattle, Dairy—
Blood-Sugar Level, Factors Influencing. R. E. Hodgson, W. H. Riddell, and J. S. Hughes ... 357-365
Grain Mixtures for, Formulation, Bulk as a Factor. L. A. Moore, C. F. Huffman, and M. M. Plum ... 789-796
Chenopodium murale, attenuation of virus of sugar-beet curly top ... 756-764

Page

Chickweed, passage of attenuated curly-top virus through, effect on virulence 755-765
Chlorine—
Content, Sap of Corn Plants, Effect of Fertilizers. N. A. Pettinger 919-931
efficacy as disinfectant of dressed ducks 914-917
Chlorophyll—
deficiencies of barley—
interrelationship 451-455
relation to other botanical characters 455-458
deficiency of tobacco—
inheritance study 477-493
recessive cause 484-493
Chromosomes—
aberrations in wheat strains, study 797, 801-830
in Grass Sorghums. A. E. Longley 317-321
CLARKE, W. S., Jr., and ANTHONY, R. D.: Growth Record of Fertilized Apple Trees Grown in Metal Cylinders 245-266
Cold, effect on Obeliscoides larvae 405-407, 418
COLE, J. R., and DEMAREE, J. B.: The Downy Spot Disease of Pecans 139-146
Colloid, separation from peat samples, procedure 99
Colloids, Dispersable Organic, in Peats, Characteristics. Wilbur L. Powers 97-111
Color in—
corn, genetical study 495-502
tobacco, inheritance study 477-493
Composts, composition, chemical and organic 105-106, 109
Conifer Seedlings, Relation to Mycorrhizae. Richard E. McArdle 287-316
Conifers, growth, relation to light intensity 227-244
Containers, types for growing alfalfa seedlings, effect on their survival 431-434
Copper-carbonate, use in control of sorghum kernel smut 10
Corn—
and Soybean Rations, Yeast and Casein Supplements to, Effect of Feeding to Rats and Swine. Charles L. Shrewsbury, Claude M. Vestal, and Sigfred M. Hauge 267-274
anthocyanin pigment, factors affecting 495-502
breeding for—
glossiness, study 167, 173
pigmentation studies 495-502
colors, genetical study 495-502
phosphoric acid content, effect of fertilizer treatments 580-581, 582-583, 586-587
plants—
absorption of chloride and sulphate ions 928-929, 930
Sap, Chlorine Content, Effect of Fertilizers. N. A. Pettinger 919-931
sap—
chlorine-potash ratio, effect of fertilizer treatments 926-928, 930
hydrogen-ion concentration as affected by chlorine content 929-930
seedlings, glossiness—
inheritance studies 167, 171-173
physical properties 168-169, 173
yields from glossy and normal plants, comparison 169
Corncob, color, source 499, 502
Cotton—
boll-weight, correlation studies 523-527
breeding, use of correlation coefficients 526-527
fertilization, selective—
differential survival of homozygotes and heterozygotes 195-196, 223, 224
factors affecting, experiments 206-215
fertilizers, irregular distribution, effects. A. L. Mehring and G. A. Cumings 559-570
fertilizing experiments 559-570
germination, relation to fertilizer distribution 562-564, 570
growth and bloom, relation to fertilizer distribution 564-565, 570
Lint Characters, Correlations and Their Practical Application. G. N. Stroman 523-527
Pima, germination behavior at low temperatures 372, 374, 375, 376-377, 379, 380
Plants, Root Constriction of, in San Joaquin Valley, California. J. W. Hubbard 39-47

Page

Cotton—Continued.
Pollen, Antagonism in. Thomas H. Kearney and George J. Harrison 191-226
pollens—
compatibility, indication by fertilization 192-194
viability tests 194
varieties, germination behavior at low temperatures, experiments 376-379, 380
yields, relation to distribution of fertilizer on 565-569, 570
Cottonseed—
age, effect on germination in the cold 379-380
Germination at Low Temperatures. C. A. Ludwig 367-380
Cream, oxidation-reduction potentials, relation to—
concentration of sugar, experiments 80, 81
sunlight 87-88
Crossbreeding—
broomcorn, for height inheritance 13-20
tobacco, in study of White Burley character 479, 480-481, 492
wheat for—
resistance to bunt 121-126
rust resistance and desirable agronomic characters 797-830
Crosses—
Interspecific, of Wheat, Variability of Strains Derived from, Cytologic and Genetic Studies. LeRoy Powers 797-831
Wheat, White Federation with Turkey, Inheritance of Resistance to Bunt, *Tilletia tritici*. Fred N. Briggs 121-126
CUMINGS, G. A., and MEHRING, A. L.: Effects on Cotton of Irregular Distribution of Fertilizers 559-570
Curly-Top Virus, Attenuated, Restoration of Virulence by Passage through *Stellaria media*. C. F. Lackey 755-765
Cyanophyceae, effect on abundance of mosquito larvae 393
Cylinders, metal, use in growing apple trees, experiments 246, 251-252
Cytologic and Genetic Studies of Variability of Wheat Strains Derived from Interspecific Crosses. LeRoy Powers 797-831
Cytological Study of Heterothallism in *Puccinia triticina*. Ruth F. Allen 733-754

Dairy Cows, Blood-Sugar Level, Factors Influencing 357-365
Damping off, seedlings, prevention by disinfectants 572-575
DANIEL, ESTHER PETERSON, and MUNSELL, HAZEL E.: The Vitamin A, B, C, and G Content of Sultanina (Thompson Seedless) and Malaga Grapes and Two Brands of Commercial Grape Juice 59-70
Darkness, effect on oxidation-reduction potential of milk 91-93, 95
Date palm. *See* Palm, date.
Day Length, Effect on Flowering Behavior of Hog Peanut. H. A. Allard 127-137
Débris in bayous, effect on mosquito breeding rates and plankton content 391-393
Dehiscence—
Linum rigidum, mechanism 21-26
of Boll of *Linum rigidum* and Related Species. A. C. Dillman and J. C. Brinsmade, jr 21-27
DEMAREE, J. B., and COLE, J. R.: The Downy Spot Disease of Pecans 139-146
DEMERITT, D. B., and MCINTYRE, A. C.: A Simple Method of Constructing Tree Volume Tables 529-539
DEMING, G. W.; ROBERTSON, D. W.; and KOONCE, DWIGHT: Inheritance in Barley 445-466
Desiccation, effect on Obeliscoides larvae 407-408, 418
Diet—
fish-meal, effect on growth of rats 592-603
protein, effect on rats and swine, experiments and results 269-273
DILLMAN, A. C., and BRINSMADE, J. C., Jr.: Dehiscence of the Boll of *Linum rigidum* and Related Species 21-27
Disinfectants, Soil, Acetic Acid and Pyroligneous Acid, Comparison with Formaldehyde. William L. Doran 571-578

Page

Disinfection, dressed ducks, experiments.. 914-918
DORAN, WILLIAM L.: Acetic Acid and Pyroligneous Acid in Comparison with Formaldehyde as Soil Disinfectants..... 571-578
Downy Spot—
Disease of Pecans. J. B. Demaree and J. R. Cole..... 139-146
of pecans—
causal fungus, study..... 141-146
economic importance and description.. 139-141
Ducklings, dressed, spoilage, cause and prevention..... 913-918
Ducks—
Dressed, Spoilage by Sliminess. W. L. Mallman..... 913-918
spoilage, cause and prevention..... 913-918
Dye, concentrated, effect on oxidation-reduction potential of milk.....77-80, 82, 90-91

Elsinoe—
on Apple and Pear. Anna E. Jenkins.... 689-700
See also *Plectodiscella piri*.
Excitement, dairy cattle, effect on blood-sugar content..... 363, 364
Exorista pyste, parasitism on larvae of strawberry leaf roller..... 556

Falcata comosa. *See* Hog peanut.
Fasting, dairy cows, effect on blood-sugar content..... 361, 364
Fat, content of milk, effect on reduction of methylene blue..... 75-77, 82
FAWCETT, H. S., and KLOTZ, L. J.: Black Scorch of the Date Palm Caused by *Thielaviopsis paradoxa*..... 155-166
FAY, A. C., and AIKINS, GLENN A.—
Effect of Light on the Reduction of Methylene Blue in Milk..... 85-95
Factors Influencing the Changes in Oxidation-Reduction Potential on the Reduction of Methylene Blue in Milk..... 71-83
Feeding, dairy cattle—
effect on blood-sugar level..... 360-361, 364
value of bulk in grain mixtures, experiments..... 789-796
Fertilization, cotton, experiments..... 191-224
Fertilizers—
apple trees, experiments..... 245-265
application to cotton plants, experiments. 559-570
Effect on—
Chlorine Content of Sap of Corn Plants. N. A. Pettinger..... 919-931
phosphoric acid content of plants, experiments..... 579-590
Irregular Distribution, Effects on Cotton. A. L. Mehring and G. A. Cumings..... 559-570
relative values for apple trees..... 261-262, 265
FICKE, C. H.; MELCHERS, L. E.; and JOHNSTON, C. O.: A Study of the Physiologic Forms of Kernel Smut (*Sphacelotheca sorghi*) of Sorghum..... 1-11
FINK, DAVID E.: Biology and Habits of the Strawberry Leaf Roller, *Ancylis comptana* (Froel.), in New Jersey..... 541-558
Fish meal—
diet, effect on growth of rats..... 592-603
protein content, nutritive value..... 596-602, 603
rations, effect on animals, experiments.... 724-732
Fish meals—
analyses..... 723
Nitrogen-Balance Studies. Burch H. Schneider..... 723-732
protein efficiency, experiments on—
rats..... 724-728, 731-732
swine..... 728-732
Vitamin A and Protein Content. L. A. Maynard, R. C. Bender, and C. M. McCay..... 591-603
Flax—
boll, dehiscence, studies..... 21-27
yellow-flowered. See *Linum rigidum*.
FLOR, H. H.: Heterothallism and Hybridization in *Tilletia tritici* and *T. laeris*..... 49-57
Flora, bacterial, in milk, effect on the form of potential time: curves..... 74-75, 82

Page

Flowering—
barley, effect on growth..... 338
Behavior, Hog Peanut, in Response to Length of Day. H. A. Allard..... 127-137
hog peanut, seasonal relationships..... 128-129
Forage, corn, phosphoric acid content, effect of fertilizer treatments..... 582-583
Forest—
mensuration, volume tables, construction method..... 529-538
Norway Pine, Light Intensity, Relation to Plant Growth. Hardy L. Shirley.. 227-244
trees, Minnesota pine forest, nomenclature..... 228
Forests, ground cover, classification under different light intensities..... 241-242, 243, 244
Formaldehyde, Comparison with Acetic Acid and Pyroligneous Acid as Soil Disinfectants. William L. Doran..... 571-578
Fraxinus americana. *See* Ash trees.
Freezing—
alfalfa seedlings, experiments. 430-435, 438-441, 444
Artificial, and Controlled Hardening, Method for Determining Comparative Hardiness in Seedling Alfalfas. George L. Peltier and H. M. Tysdal..... 429-444
Fungi, species forming mycorrhizae, list.... 296
Fusarium—
martii phaseoli—
virulence..... 175
See also Root rot, dry, of beans.
rot, development on carrots in storage. 896-906, 911

Genetics, wheat, studies..... 797-830
Germination—
cotton, relation to fertilizer distribution.. 562-564, 570
Cottonseed at Low Temperatures—
C. A. Ludwig..... 367-380
test methods..... 367-370
effects of soil disinfectants on..... 575-576
Glossy Character (gl) in Maize and Its Linkage Relations. H. L. Thomas..... 167-173
Glucose, feeding to dairy cows, effect on blood-sugar content..... 361-363, 364
Goose-foot, nettle-leafed, attenuation of curly-top virus..... 756-765
Gossypium nanking, germination behavior at low temperatures..... 373, 375, 376, 378, 379, 380
Grain—
Mixtures, Formulation for Dairy Cattle, Bulk as a Factor. L. A. Moore, C. F. Huffman, and M. M. Plum..... 789-796
phosphoric acid content, effect of fertilizer treatments..... 580-587
Grape—
diet, effect on longevity of rats..... 60, 61-63, 67-69
Juice—
Commercial, and Sultanina (Thompson Seedless) and Malaga Grapes, Vitamin A, B, C, and G Content. Esther Peterson Daniel and Hazel E. Munsell..... 59-70
diet, effect on guinea pigs..... 64-66, 69
vitamin content, experiments..... 59-70
Grapes—
Malaga and Sultanina (Thompson Seedless), and Commercial Grape Juice, Vitamin A, B, C, and G Content. Esther Peterson Daniel and Hazel E. Munsell..... 59-70
vitamin content, experiments..... 59-70
Grass Sorghums, Chromosomes in. A. E. Longley..... 317-321
Gray-mold rot of carrots, study..... 886-895, 898-906, 907, 910
GREEN, E. L.; HORSFALL, J. G.; and KERTESZ, Z. I.: Some Effects of Root Rot on the Physiology of Peas..... 833-848
GREEN, JESSE R.: Chemical and Physical Properties of Petroleum Spray Oils..... 773-787
Green Manure, Vetch—
Decomposition in Relation to the Surrounding Soil. Harry Humfeld and Nathan R. Smith..... 113-120
fresh and decomposing, contents..... 115-120

Page

GROVES, A. B.: A Photographic Light Box for Use in Agricultural Research 467-475
Growth—
Apple Trees, Fertilized, in Metal Cylinders, Records. R. D. Anthony and W. S. Clarke, jr 245-266
Curve in Barley—
Merritt N. Pope 323-341
Relation to Catalase Activity. Merritt N. Pope 343-355
Guinea pigs, infection with *Obeliscoides cuniculi*, experiments 414-417, 418

HALVERSON, J. O., and SHERWOOD, F. W.: The Distribution of Vitamin B Complex and Its Components in the Peanut 849-860
HARRISON, GEORGE J., and KEARNEY, THOMAS H.: Pollen Antagonism in Cotton 191-226
HAUGE, SIGFRED M.; SHREWSBURY, CHARLES L.; and VESTAL, CLAUDE M.: The Effect of Yeast and Casein Supplements to Corn and Soybean Rations When Fed to Rats and Swine 267-274
Hay—
phosphoric acid content, effect of fertilizer treatments 583-584, 585-586
soybean, phosphoric acid content, effect of fertilizer treatments 583, 584-585
Heat, effect on Obeliscoides larvae 407, 418
Height in Broomcorn, Inheritance. John B. Sieglinger 13-20
HENIKA, F. S.: The Inheritance of the White Burley Character in Tobacco 477-493
Herbage, mixed, phosphoric acid treatment of, effect 587-588
Herbs in Minnesota pine forest, nomenclature 226-229
Heredity—
and Nutrition, Effects upon Litter Size in Swine and Rats. H. P. Morris and Don W. Johnson 511-521
See also Inheritance.
Heterodera radicicola, cause of tomato root-knot 275
Heterothallism—
and Hybridization in *Tilletia tritici* and *T. laevis*. H. H. Flor 49-57
in *Puccinia triticina*, Cytological Study. Ruth F. Allen 733-754
in *Tilletia tritici*, experimental results 52-53
Heterozygotes, cotton plants, differential survival in fertilization 195-196
Hibernation, strawberry leaf roller, description 552-554, 557
Hicoria pecan. *See* Pecan; Pecans.
HODGSON, R. E.; RIDDELL, W. H.; and HUGHES, J. S.: Factors Influencing the Blood-Sugar Level of Dairy Cattle 357-365
Hog peanut—
flowers, description 127-129, 135
Flowering Behavior in Response to Length of Day. H. A. Allard 127-137
Holcus sorghum. *See* Broomcorn.
Homozygotes, cotton plants, differential survival in fertilization 195-196, 202-206, 217-219, 223, 224
HORSFALL, J. G.; KERTESZ, Z. I.; and GREEN, E. L.: Some Effects of Root Rot on the Physiology of Peas 833-848
HUBBARD, J. W.: Root Constriction of Cotton Plants in the San Joaquin Valley of California 39-47
HUFFMAN, C. F.; MOORE, L. A.; and PLUM, M. M.: Bulk as a Factor in Formulating Grain Mixtures for Dairy Cattle 789-796
HUGHES, J. S.; HODGSON, R. E.; and RIDDELL, W. H.: Factors Influencing the Blood-Sugar Level of Dairy Cattle 357-365
HUMFELD, HARRY, and SMITH, NATHAN R.: The Decomposition of Vetch Green Manure in Relation to the Surrounding Soil 113-120
Humidity—
effect on shriveling of carrots 906-907, 911
relation to fungous infection of carrots in storage 868-869, 885, 892-893, 909, 910, 911

Page

Hybridization and Heterothallism in *Tilletia tritici* and *T. laevis*. H. H. Flor 49-57
Hydrogen-Ion Concentration of—
Soil, Effect on Growth of Bean and Its Susceptibility to Dry Root Rot. Walter H. Burkholder 175-181
water, relation to presence of Anopheles larvae 304-306, 309
Hypochlorite, efficiency in controlling sliminess of ducklings 915-916, 918

Ice, specific heat, table 680
IMMER, F. R.—
A Study of Sampling Technic with Sugar Beets 633-647
Size and Shape of Plot in Relation to Field Experiments with Sugar Beets 649-668
Infection of—
guinea pigs, with rabbit stomach worms, experiments 414-418
rabbits, with stomach worms, experiments 409-413
Inheritance—
glossiness in corn seedlings, study 167, 171-173
Height in Broomcorn. John B. Sieglinger 13-20
in Barley. D. W. Robertson, G. W. Deming, and Dwight Koonce 445-466
litter size in swine and rats, study 511-520
Resistance to Bunt, *Tilletia tritici*, in Crosses of White Federation with Turkey Wheats. Fred N. Briggs 121-126
White Burley Character in Tobacco. F. S. Henika 477-493
Inoculation—
carrots, with storage-rot fungi, experiments 884-911
oat varieties, with covered and loose smuts, study 148-150
pecan leaves, with *Cercosporella caryigena* 144-145
sugar beets, with *Phoma betae*, temperature studies 29-37
Insecticides, injury to foliage, study 773-786
Irrigation, effect on root constriction of cotton plants 40-47

JENKINS, ANNA E.: Elsinoe on Apple and Pear 689-700
JENKINS, MERLE T.: An Additional Pair of Factors Affecting Anthocyanin Pigment in Maize 495-502
JOHNSON, DON W., and MORRIS, H. P.: Effects of Nutrition and Heredity upon Litter Size in Swine and Rats 511-521
Johnson grass, chromosome numbers, study 317, 318, 320
JOHNSTON, C. O.; MELCHERS, L. E.; and FICKE, C. H.: A Study of the Physiologic Forms of Kernel Smut (*Sphacelotheca sorghi*) of Sorghum 1-11
JONES, LINUS H.: The Effect of Environment on the Nematode of the Tomato Gall 275-285

Karyotin, Marquillo wheat, extrusion 804-805, 829
KEARNEY, THOMAS H., and HARRISON, GEORGE J.: Pollen Antagonism in Cotton 191-226
Kernel Smut—
(*Sphacelotheca sorghi*) of Sorghum, Physiologic Forms, Study. L. E. Melchers, C. H. Ficke, and C. O. Johnston 1-11
physiologic forms, key for identification 6
KERTESZ, Z. I.; HORSFALL, J. G.; and GREEN, E. L.: Some Effects of Root Rot on the Physiology of Peas 833-848
KIFER, HILDA BLACK, and MUNSELL, HAZEL E.: Vitamin Content of Three Varieties of Spinach 767-771
KLOTZ, L. J., and FAWCETT, H. S.: Black Scorch of the Date Palm Caused by *Thielaviopsis paradoxa* 155-166
KOONCE, DWIGHT; ROBERTSON, D. W.; and DEMING, G. W.: Inheritance in Barley 445-466

LACKEY, C. F.: Restoration of Virulence of Attenuated Curly-Top Virus by Passage through *Stellaria media* 755-765

Page

LAURITZEN, J. I.: Development of Certain Storage and Transit Diseases of Carrot.. 861-912
Leaf Roller, Strawberry, *Ancylis comptana* (Froel.), in New Jersey, Biology and Habits. David E. Fink.. 541-558
Leaf rust, of wheat. See *Puccinia triti-ina.*
Lemna spp., effect on abundance of mosquito larvae.. 393-394
Light—
artificial, effect on—
flowering of hog peanut.. 133-135, 137
oxidation-reduction potential of milk.. 93-94, 95
box, photographic—
construction and fitting.. 470-474
Use in Agricultural Research. A. B. Groves.. 467-475
effect on—
Obeliscoides larvae.. 408
Reduction of Methylene Blue in Milk. Glenn A. Aikins and A. C. Fay.. 85-95
Intensity, Relation to Plant Growth in a Virgin Norway Pine Forest. Hardy L. Shirley.. 227-244
measurements, pine forest, correlation studies.. 233-242, 243
Lignin, in peat colloids, base-exchange capacity.. 106-109
Lima beans. *See* Beans, Lima.
Linum—
rigidum—
and Related Species, Boll of, Dehiscence. A. C. Dillman and J. C. Brinsmade, jr.. 21-27
dehiscence, mechanism.. 21-25
spp., dehiscence, studies.. 21-27
Litter Size, Swine and Rats, Effects of Nutrition and Heredity. H. P. Morris and Don W. Johnson.. 511-521
LONGLEY, A. E.: Chromosomes in Grass Sorghums.. 317-321
LUDWIG, C. A.: The Germination of Cottonseed at Low Temperatures.. 367-380

Maize—
Anthocyanin Pigment, Factors Affecting, Additional Pair. Merle T. Jenkins.. 495-502
Glossy Character (gl_3) and Its Linkage Relations. R. L. Thomas.. 167-173
MALLMANN, W. L.: The Spoilage of Dressed Ducks Caused by Sliminess.. 913-918
Manure—
effect on chlorine content of corn sap, study.. 919-923, 924-926, 927, 930
See also Green manure.
MAYNARD, L. A.; BENDER, R. C.; and MCCAY, C. M.: Vitamin A and Protein Content of Various Fish Meals.. 591-603
MCARDLE, RICHARD E.: The Relation of Mycorrhizae to Conifer Seedlings.. 287-316
MCCAY, C. M.; MAYNARD, L. A.; and BENDER, R. C.: Vitamin A and Protein Content of Various Fish Meals.. 591-603
MCINTYRE, A. C., and DEMERITT, D. B.: A Simple Method of Constructing Tree Volume Tables.. 529-539
Meals, fish—
protein efficiency, experiments on—
rats.. 724-728, 731-732
swine.. 728-732
Vitamin A and Protein Content. L. A. Maynard, R. C. Bender, and C. M. McCay.. 591-603
MEHRING, A. L., and CUMINGS, G. A.: Effects on Cotton of Irregular Distribution of Fertilizers.. 559-570
MELCHERS, L. E.; FICKE, C. H.; and JOHNSTON, C. O.: A Study of the Physiologic Forms of Kernel Smut (*Sphacelotheca sorghi*) of Sorghum.. 1-11
Mendelian characters, of barley, inheritance studies.. 448-451, 465-466
Menhaden meal, protein efficiency, study.. 723-732
Metal Cylinders, Growing Fertilized Apple Trees in, for Growth Record. R. D. Anthony and W. S. Clarke, jr.. 245-266

Page

Methylene blue in milk—
effect on form of potential time: curves, experiments.. 72-74
Reduction—
Effect of Light on. Glenn A. Aikins and A. C. Fay.. 85-95
Factors Influencing Changes in Oxidation-Reduction Potential. A. C. Fay and Glenn A. Aikins.. 71-83
Microorganisms, number in decomposing green manure, study.. 113-116, 118-119, 120
Microsporogenesis, wheat, studies.. 802-830
Milk—
Methylene Blue in, Reduction—
Effect of Light. Glenn A. Aikins and A. C. Fay.. 85-95
Factors Influencing Changes in Oxidation-Reduction Potential. A. C. Fay and Glenn A. Aikins.. 71-83
oxidation-reduction potential, relation to—
alternate light and darkness.. 91-92
artificial light.. 93-94, 95
sunlight.. 87
yield, dairy cows, effect on blood-sugar level.. 359-360, 364
Moisture, effect on tomato root-knot nematode.. 276-279, 281-284
MOORE, L. A.; HUFFMAN, C. F.; and PLUM, M. M.: Bulk as a Factor in Formulating Grain Mixtures for Dairy Cattle.. 789-796
MORRIS, H. P., and JOHNSON, DON W.: Effects of Nutrition and Heredity upon Litter Size in Swine and Rats.. 511-521
Mosquito larvae, dissection, studies.. 396-398, 399
Mosquitoes—
Anopheles, Breeding, Associated Factors. G. H. Bradley.. 381-399
See also Anopheles.
MUNSELL, HAZEL E., and—
DANIEL, ESTHER PETERSON: The Vitamin A, B, C, and G Content of Sultanina (Thompson Seedless) and Malaga Grapes and Two Brands of Commercial Grape Juice.. 59-70
KIFER, HILDA BLACK: Vitamin Content of Three Varieties of Spinach.. 767-771
Muriate of potash, effect on chlorine content of corn sap, experiments.. 919-927, 930
Mycorrhizae—
formation on spruce and pine under controlled conditions.. 297-305
function in conifer development, discussion.. 287-288, 291-293, 310-314
Relation to Conifer Seedlings. Richard E. McArdle.. 287-316
species found on pine and spruce, list.. 295
types and occurrence.. 288, 295
Mycorrhizal mantle, development on conifers, study.. 305-310
Mycosphaerella caryigena—
description and pathogenicity.. 141-143
growth on artificial media, physiological studies.. 145-146
n. comb., taxonomic position and technical description.. 143

Nematode, Tomato Gall, Effect of Environment. Linus H. Jones.. 275-283
Nematodes, resistance to aniline stain.. 408-409
New Jersey, Strawberry Leaf Roller, *Ancylis comptana* (Froel.), in, Biology and Habits. David E. Fink.. 541-558
Nitric nitrogen, content of soil at various depths.. 117, 119
Nitrogen—
absorption by seedlings, aid of mycorrhizae, discussion.. 311, 314
Balance Studies with Various Fish Meals. Burch H. Schneider.. 723-733
content of—
decomposing green manure.. 116-117, 119
peat colloid, determination.. 102
influence on phosphoric acid content of barley.. 581-582
use in growing apple trees.. 248-250, 252, 253-256, 257-263, 265

Page

Norway Pine Forest, Virgin, Light Intensity, Relation to Plant Growth. Hardy L. Shirley 227-244
Nutrition and Heredity, Effects upon Litter Size in Swine and Rats. H. P. Morris and Don W. Johnson 511-521

Oak, red, volume tables, construction, simple method 530-538
Oats—
Fulghum, susceptibility to newly discovered race of *Ustilago levis* 147, 150, 152
phosphoric acid content, effect of fertilizer treatments 580-581
Red—
Ustilago levis and *U. avenae* on, Physiological Races. George M. Reed and T. R. Stanton 147-153
varieties, reactions to inoculations of covered and loose smuts 148-150, 152
Obeliscoides—
cuniculi—
infection of guinea pigs 414-418
infection of rabbits, experiments 409-413
Rabbit Stomach Worm, Life History. Joseph E. Alicata 401-419
larvae—
development 402-404
reactions, experiments 404-418
Oestrus, dairy cows, effect on blood-sugar content 363
Oils, petroleum spray—
analysis methods and results 773-776, 777
Chemical and Physical Properties. Jesse R. Green 773-787
properties injurious to plants, study 781-786
Olive-Tubercle Organism—
Bacterium savastanoi, Cause of Canker of Ash Trees. Nellie A. Brown 701-722
tests and comparisons with ash-canker organism 708-721, 722
Oxidation-Reduction Potential, Changes in, Relation to Reduction of Methylene Blue in Milk, Factors Influencing. A. C. Fay and Glenn A. Atkins 71-83

Pack, Dean A., and Tompkins, C. M.: Effect of Temperature on Rate of Decay of Sugar Beets by Strains of *Phoma betae*. 29-37
Palm, Date—
Black Scorch—
Caused by *Thielaviopsis paradoxa*. L. J. Klotz and H. S. Fawcett 155-166
symptoms 156-161
varieties, susceptibility to black scorch 161-163
Parasites, strawberry leaf roller, description 554-556, 557
Peanut—
hog. *See* Hog peanut.
kernel parts, vitamin B content, experiments 850-860
Vitamin B Complex and Its Components, Distribution. F. W. Sherwood and J. O. Halverson 849-860
Pear and Apple, Elsinoe on. Anna E. Jenkins 689-700
Peas—
chemical changes due to root rot, study 834, 838-847
Physiology, Effects of Root Rot. J. G. Horsfall, Z. I. Kertesz, and E. L. Green. 833-848
ripening and quality, effect of root rot, study 834, 835-847
Root Rot, Effects on Physiology. J. G. Horsfall, Z. I. Kertesz, and E. L. Green. 833-848
Peat colloids—
amount and absorptiveness 100
organic composition and base-exchange capacity 100-102, 109
Peats, Colloids in, Dispersable Organic, Characteristics. Wilbur L. Powers 97-111
Peaty materials, base-exchange capacity 102-103
Pecan, varieties susceptible to downy spot 140
Pecans, Downy Spot Disease. J. B. Demaree and J. R. Cole 139-146

Page

Peltier, George L., and Tysdal, H. M.: A Method for the Determination of Comparative Hardiness in Seedling Alfalfas by Controlled Hardening and Artificial Freezing 429-444
Penicillium rot, development on carrots in storage, experiments 896-900, 911
Petroleum Spray Oils, Chemical and Physical Properties. Jesse R. Green 773-787
Pettinger, N. A.: Effect of Fertilizers on the Chlorine Content of the Sap of Corn Plants 919-931
Phaseolus vulgaris. *See* Bean; Beans.
Phoenix dactylifera. *See* Palm, date.
Phoma betae—
inoculation of sugar beets, experiments 29-37
Strains, Decay of Sugar Beets, Effect of Temperature on Rate. C. M. Tompkins and Dean A. Pack 29-37
Phosphate, rock, effect on—
chlorine content of corn sap, study 919-921
phosphoric acid content of crops 582-583
Phosphates, influence on phosphoric acid content of—
barley 583, 586
corn forage 582-583
Plant. A. W. Blair and A. L. Prince 579-590
rape 583, 585
rye 583-584
soybean hay 583, 584, 585
Phosphoric Acid—
Content of Plant, Influence of Phosphates. A. W. Blair and A. L. Prince. 579-590
effect on mixed herbage 587-588
influence on the phosphoric acid content of barley 581-582
Phosphorus, use in growing apple trees 248-250, 253-254, 257-263, 265
Photographic Light Box, Use in Agricultural Research. A. B. Groves 467-475
Photography, value in agricultural research 468-470
Photoperiodism experiments with hog peanut 127-137
Pigment, Anthocyanin, in Maize, an Additional Pair of Factors Affecting. Merle T. Jenkins 495-502
Pigmentation, tobacco, inheritance study 477-493
Pigs, litter size, factors affecting 516-520
Pine forest, light measurements, correlation studies 233-242, 243
Pine Forest, Norway Virgin, Light Intensity, Relation to Plant Growth. Hardy L. Shirley 227-244
Pines, mycorrhizae of, types and formation 296-297
Plankton—
content of water, factors affecting 382-388, 392-393, 396
genera, occurrence in Anopheles breeding and nonbreeding waters 387-388
Plant—
cell walls, disintegration by phytopathogenic bacteria 625-631, 632
Growth, in Relation to Light Intensity in a Virgin Norway Pine Forest. Hardy L. Shirley 227-244
materials, composted—
composition 105-106, 109
loss of dry organic matter 103-105
Phosphoric Acid Content, Influence of Phosphates. A. W. Blair and A. L. Prince 579-590
sap, sucrose content, determination 671-674, 686
Tissue, Bound Water in, Methods of Determining. J. D. Sayre 669-683
Plants—
dry weight, effect of soil disinfectants 572, 576-577, 578
injury by spray oils, study 773-786
Plectodiscella piri—
history and distribution on apple and pear 689-690
morphology 690-691, 697
taxonomy 691-695, 697

Page

PLUM, M. M.; MOORE, L. A.; and HUFFMAN, C. F.: Bulk as a Factor in Formulating Grain Mixtures for Dairy Cattle.... 789-796

Pollen—
Antagonism in Cotton—
Thomas H. Kearney and George J. Harrison.... 191-226
hypothesis.... 219-223, 224
relation to consanguinity, experiments.... 215-219
tubes, cotton, differential growth rate, cause of selective fertilization.... 196-206

Pollination, of cotton, experiments and results.... 191-224

POPE, MERRITT N.—
Catalase Activity in Relation to the Growth Curve in Barley.... 343-355
The Growth Curve in Barley.... 323-341

Potash, influence on phosphoric acid content of barley.... 581-582

Potassium, use in growing apple trees.... 248-250, 253, 255, 257-263, 265

Potatoes, phosphorus content, effect of fertilizer treatments.... 588-589

POWERS, LEROY: Cytologic and Genetic Studies of Variability of Strains of Wheat Derived from Interspecific Crosses.... 797-831

POWERS, WILBUR L.: Characteristics of Dispersable Organic Colloids in Peats.... 97-111

Precipitation, influence on—
abundance of mosquito larvae.... 382-384
plankton organisms in water.... 385-386

PRINCE, A. L., and BLAIR, A. W.: The Influence of Phosphates on the Phosphoric Acid Content of the Plant.... 579-590

Protein—
and Vitamin A Content, Various Fish Meals. L. A. Maynard, R. C. Bender, and C. M. McCay.... 591-603
casein, feeding to pigs and rats.... 267-273
content, fish meal—
experiments.... 724-732
nutritive value.... 596-602, 603

Protozoa, number in decomposing green manure, study.... 113-116, 119, 120

Puccinia—
graminis tritici, resistance of Marquillo wheat.... 797, 801
triticina—
fertile infection on Thalictrum, study.... 745-749, 750-753
Heterothallism, Cytological Study. Ruth F. Allen.... 733-754
invasion of Thalictrum leaf.... 735-740, 741-749, 752-753
isolated infection on Thalictrum, study.... 741-745, 750
spermogonia and receptive hyphae, study.... 738-740, 749-753

Pyroligneous Acid and Acetic Acid, Comparison with Formaldehyde as Soil Disinfectants. William L. Doran.... 571-578

Quercus borealis maxima, volume tables, construction, simple method.... 530-538

Rabbit Stomach Worm, *Obeliscoides cuniculi*. Life History. Joseph E. Alicata.... 401-419

Rabbits, infection with *Obeliscoides cuniculi*, experiments.... 404, 409-413, 418

Raspberry, damage by strawberry leaf roller.... 542, 557

Rations—
Corn and Soybean, Yeast and Casein Supplements to, Effect of Feeding to Rats and Swine. Charles L. Shrewsbury, Claude M. Vestal, and Sigfred M. Hauge.... 267-274
grain, for dairy cattle, value of bulk, experiments.... 789-796

Rats—
and Swine, Litter Size, Effects of Nutrition and Heredity. H. P. Morris and Don E. Johnson.... 511-521
feeding with—
peanuts, vitamin study.... 850-860
yeast and casein supplements to corn and soybean rations, effect.... 269-271, 273

Page

Rats—Continued.
growth on protein and protein-deficient diet, experiments.... 269-271
litter size, factors affecting.... 513-516, 519-520

Red pine seedlings, damping off, effect of soil treatment with acetic acid.... 575, 577-578

REED, GEORGE M., and STANTON, T. R.: Physiologic Races of *Ustilago levis* and *U. avenae* on Red Oats.... 147-153

Research, Agricultural, Use of Photographic Light Box. A. B. Groves.... 467-475

Rhizopus—
infection of sweetpotatoes and carrots, comparison.... 876-877
nigricans, growth on carrots in storage, experiments.... 861, 863, 870-877, 908-909
soft rot, development on carrots, experiments.... 861-863, 870-879, 896-902, 907-911
tritici, growth on carrots in storage, experiments.... 861, 863, 870-879, 908-909

Rice polish, extract, feeding to rats, experiments.... 854-859

RIDDELL, W. H.; HODGSON, R. E.; and HUGHES, J. S.: Factors Influencing the Blood-Sugar Level of Dairy Cattle.... 357-365

ROBERTSON, D. W.; DEMING, G. W.; and KOONCE, DWIGHT: Inheritance in Barley.... 445-466

Root—
Constriction, Cotton Plants in San Joaquin Valley, California. J. W. Hubbard.... 39-47
growth, relation to soil temperature.... 422

Root Rot—
Dry, of Beans, Relation to Hydrogen-Ion Concentration of Soil. Walter H. Burkholder.... 175-181
of peas—
causal organisms.... 833
effect on ripening and quality, study.... 834, 835-847
Effects on Physiology. J. G. Hersfall, Z. I. Kertesz, and K. L. Green.... 833-848

Roots, conifer, association with mycorrhizae, study.... 287-314

Rumen, cattle, activity in dealing with grain mixtures.... 789-796

Rye, phosphoric acid content, effect of fertilizer treatments.... 583-584, 588-589

Sampling Technic, Study with Sugar Beets. F. R. Immer.... 633-647

San Joaquin Valley, California, Cotton Plants, Root Constriction. J. W. Hubbard.... 39-47

Sap, Corn Plants, Chlorine Content, Effect of Fertilizers. N. A. Pettinger.... 919-931

SAYRE, J. D.: Methods of Determining Bound Water in Plant Tissue.... 669-688

SCHNEIDER, Burch H.: Nitrogen-Balance Studies with Various Fish Meals.... 723-733

Sclerotinia—
sclerotiorum, development on carrots in storage, experiments.... 861, 863-870, 907-908, 910-911
soft rot, development on carrots.... 861-870, 896, 907-909

Seed germination, effects of soil disinfection.... 575-576, 577-579

Seedlings—
Alfalfa, Comparative Hardiness in, Method for Determination by Controlled Hardening and Artificial Freezing. George L. Peltier and H. M. Tysdal.... 429-444
association with mycorrhizae, benefits.... 310-314
damping off, effect of soil disinfectants.... 573-579

SHERWOOD, F. W., and HALVERSON, J. O.: The Distribution of Vitamin B Complex and Its Components in the Peanut.... 849-860

SHIRLEY, HARDY L.: Light Intensity in Relation to Plant Growth in a Virgin Norway Pine Forest.... 227-244

SHREWSBURY, CHARLES L.; VESTAL, CLAUDE M.; and HAUGE, SIGFRED M.: The Effect of Yeast and Casein Supplements to Corn and Soybean Rations When Fed to Rats and Swine.... 267-274

Page

Shrubs, in Minnesota pine forest, nomenclature 228
SIEGLINGER, JOHN B.: Inheritance of Height in Broomcorn 13-20
Silviculture, relation to light studies 242-243, 244
Skim milk, oxidation-reduction potentials, relation to sunlight 88-91
Sliminess, Cause of Spoilage of Dressed Ducks. W. L. Mallmann 913-918
Slimy soft rot. *See* Bacterial soft rot.
SMITH, ALFRED: Seasonal Subsoil Temperature Variations 421-428
SMITH, NATHAN R., and HUMFELD, HARRY: The Decomposition of Vetch Green Manure in Relation to the Surrounding Soil 113-120
SMITH, ORA: Relation of Temperature to Anthesis and Blossom Drop of the Tomato, together with a Histological Study of the Pistils 183-190
Smut—
covered, physiologic races on red oats 147-149, 150, 152
loose, physiologic races on red oats 147, 149-150, 151-152
wheat—
heterothallism and hybridization, studies 49-57
sporidia, formation of, description 51-53
Sodium—
chloride, efficiency in controlling sliminess of dressed ducklings 917-918
hypochlorite, efficiency in controlling sliminess of dressed ducklings 915-916, 918
Soil—
bacteria, number in decomposing green manure, study 113, 115, 118-119, 120
desiccated, effect on tomato root-knot nematode 280-281
Disinfectants, Acetic Acid and Pyroligneous Acid, Comparison with Formaldehyde. William L. Doran 571-578
flooded, effect on tomato root-knot nematode 280
heterogeneity, effect on sugar content of beets 646, 647
Hydrogen-Ion Concentration, Effect on Growth of Bean and Its Susceptibility to Dry Root Rot. Walter H. Burkholder 175-181
manured, hydrogen-ion concentration 117, 119
microorganisms, number in decomposing green manure, study 113-116, 118-119, 120
moisture, effect on—
survival of alfalfa seedlings during freezing 439-440
tomato root-knot nematode 276-279, 280, 281-284
nitrogen, parts in decomposing green manure, study 116-117, 119
temperature—
effect on root growth 422
effect on survival of alfalfa seedlings during freezing 438-441
seasonal variations 421-427
Soils, fertilizer treatments, relation to phosphoric acid content of plants 579-590
Sorghum—
halepensis, chromosome study 317-321
kernel smut—
control by copper-carbonate dust method 10
(*Sphacelotheca sorghi*), Study of Physiologic Forms. L. E. Melchers, C. H. Ficke, and C. O. Johnston 1-11
vulgare. *See* Broomcorn.
Sorghums—
annual, chromosome numbers, study 317-318, 320, 321
Grass, Chromosomes in. A. E. Longley 317-321
Inoculation with *Sphacelotheca sorghi*, studies 3-6, 10
varieties susceptible to kernel smut 3-6, 10

Page

Soybean—
and Corn Rations, Yeast and Casein Supplements to, Effect of Feeding to Rats and Swine. Charles L. Shrewsbury, Claude M. Vestal, and Sigfred M. Hauge 267-274
hay, phosphoric acid content, effect of fertilizer treatments 583, 584
Soybeans, cooked, superiority to uncooked 270-271, 272
Sphacelotheca sorghi—
Kernel Smut of Sorghum, Physiologic Forms, Study. L. E. Melchers, C. H. Ficke, and C. O. Johnston 1-11
morphologic characters 7-9, 10
physiologic—
forms, distribution and importance 9-10
forms, key for identification 6
specialization, experiments and results 3-6, 10
Spinach, Vitamin Content of Three Varieties. Hilda Black Kifer and Hazel E. Munsell 767-771
Spray Oils, Petroleum, Chemical and Physical Properties. Jesse R. Green 773-787
Spruce, mycorrhizae of, types and formation 295-296
Squab ducklings, spoilage, cause and prevention 913-918
STANTON, T. R., and REED, GEORGE M.: Physiologic Races of *Ustilago levis* and *U. avenae* on Red Oats 147-153
Stellaria media, Restoration of Virulence of Attenuated Curly-Top Virus by Passage through. C. F. Lackey 755-765
Stomach worm—
larvae, infective, experiments 404-409
Obeliscoides cuniculi, of Rabbit, Life History. Joseph E. Alicata 401-419
Strawberry—
damage by strawberry leaf roller 542, 557
Leaf Roller—
Ancylis comptana (Froel.), in New Jersey, Biology and Habits. David E. Fink 541-555
control by parasites 554-556, 557
distribution and nature of injury 542
life cycle, description 543-554
STROMAN, G. N.: Correlations of Certain Lint Characters in Cotton and Their Practical Application 523-527
Subsoil and Temperature Variations, Seasonal. Alfred Smith 421-428
Sucrose—
in plant sap, determination, experiments 671-674, 686
solutions, freezing-point depression, determination 674-676, 686
Sugar—
concentration of, effect on oxidation-reduction potentials in cream 80-81, 82
content, beets, determination by sampling, method 633-647, 658-661, 663, 664-668
Sugar beets—
curly-top virus, virulence, attenuation and restoration 755-765
Decay—
by Strains of *Phoma betae*, Effect of Temperature on Rate. C. M. Tompkins and Dean A. Pack 29-37
effect of temperature on 29-37
Field Experiments, Effect of Size and Shape of Plot. F. R. Immer 649-668
in storage, decay, experiments 30-32
inoculation with *Phoma betae*, temperature studies 29-37
juice, purity, analysis 661-667, 668
Plots, Size and Shape, Relation to Field Experiments. F. R. Immer 649-668
sampling technic—
analysis of variance 634-647, 651-668
Study. F. R. Immer 633-647
weight—
analysis of variance 652-653
relation to sugar content, study 633-640, 666
yield, analysis of variance 651-658, 665-666

Page

Sunlight—
effect on reduction of methylene blue in milk 86–91, 93, 94–95
See also Light.
Superphosphate, effect on—
chlorine content of corn sap, study 919–921
phosphoric acid content of crops 583–586
Sweetpotatoes, infected with Rhizopus, comparison with infected carrots 876–877
Swine—
and Rats, Litter Size, Effects of Nutrition and Heredity. H. P. Morris and Don W. Johnson 511–521
feeding with fish meal, experiments 728–732
feeding with yeast and casein supplements to corn and soybean rations, effect 272–273
growth on protein and protein-deficient diet, experiments, and results 272–273
litter size, factors affecting 516–520

Taproots, cotton, root constriction 42–47
Temperature—
effect on—
etiolation of tobacco plants 490
hibernating larvae and pupae of strawberry leaf roller 552–554, 557
Rate of Decay of Sugar Beets by Strains of *Phoma betae*. C. M. Tompkins and Dean A. Pack 29–37
tomato root-knot nematode 276–278
influence on—
abundance of mosquito larvae 382–384
infection and decay of carrots in storage 865–911
plankton organisms in water 385–386
Relation to Tomato Anthesis and Blossom Drop, together with a Histological Study of the Pistils. Ora Smith 183–190
soil, seasonal variations 421–427
Variations, Seasonal, of Subsoil. Alfred Smith 421–428
Temperatures, Low, Effect on Cottonseed Germination. C. A. Ludwig 367–380
Thalictrum leaf—
inoculation with *Puccinia triticina* 735–736
invasion by *Puccinia triticina* mycelium 736–740, 741–749
Thielaviopsis paradoxa—
Cause of Black Scorch of Date Palm. L. J. Klotz and H. F. Fawcett 155–166
control 164–165
description 163–164, 165
host plants and range 165
Thomas, H. L.: The Glossy Character (gl$_3$) in Maize and Its Linkage Relations 167–173
Thresher, Injury to Baby Lima Beans. H. A. Borthwick 503–510
Tilletia—
levis—
and *T. tritici*, Heterothallism and Hybridization in. H. H. Flor 49–57
physiologic forms, hybridization 53–57
tritici—
and *T. levis*, Heterothallism and Hybridization in. H. H. Flor 49–57
physiologic forms, hybridization 53–57
Timber volume tables, construction, simple method 529–538
Tissue, Plant, Bound Water in, Methods of Determining. J. D. Sayre 669–688
Tobacco—
breeding for inheritance of White Burley character 479–493
chlorophyll deficiency, inheritance study 477–493
seed, weight, effect on white genotypes 487–490, 493
White Burley Character, Inheritance. F. S. Henika 477–493
Tomato—
Anthesis and Blossom Drop, Relation to Temperature, together with a Histological Study of the Pistils. Ora Smith 183–190
blossom drop, relation to temperature, experiments 183–190
Gall, Nematode—
Effect of Environment. Linus H. Jones 275–285
nature and population 275–276

Page

Tomato—Continued.
galls, decay, relation to soil moisture 281–283, 284
pistils, histological study 187–190
root-knot nematode, environmental effects, study 275–284
Tompkins, C. M., and Pack, Dean A.: Effect of Temperature on Rate of Decay of Sugar Beets by Strains of *Phoma betae* 29–37
Tree Volume Tables, Construction, Simple Method. D. B. Demeritt and A. C. McIntyre 529–539
Trees—
Apple, Fertilized, Growing in Metal Cylinders, Growth Record. R. D. Anthony and W. S. Clarke, jr 245–266
Ash, Canker of, Production by Variety of Olive Tubercle Organism, *Bacterium savastanoi*. Nellie A. Brown 701–722
classification by diameter and height 530–532
diameter axis, plotting for volume tables 533–534
height axis, plotting for volume tables 534
in Minnesota forest, nomenclature 228
volume axis, plotting for volume tables 534–538
Tysdal, H. M., and Peltier, George L.: A Method for the Determination of Comparative Hardiness in Seedling Alfalfas by Controlled Hardening and Artificial Freezing 429–444

Ustilago—
avenae and *U. levis*, Physiologic Races on Red Oats. George M. Reed and T. R. Stanton 147–153
tritici, physiologic races on red oats 147, 149–150, 151–152

Vestal, Claude M.; Shrewsbury, Charles L.; and Hauge, Sigfred M.: The Effect of Yeast and Casein Supplements to Corn and Soybean Rations When Fed to Rats and Swine 267–274
Vetch Green Manure, Decomposition in Relation to the Surrounding Soil. Harry Humfeld and Nathan R. Smith 113–120
Virulence, Curly-Top Virus, Attenuated, Restoration by Passage through *Stellaria media*. C. F. Lackey 755–765
Virus, Attenuated Curly-Top, Virulence Restoration by Passage through *Stellaria media*. C. F. Lackey 755–765
Vitamin A—
and Protein Content, Various Fish Meals. L. A. Maynard, R. C. Bender, and C. M. McCay 591–603
content—
fish meal, experiments 593–596, 602–603
of grapes and grape juice, experiments 60, 69
determination in three varieties of spinach 768–769, 771
Vitamin B, C, and G Content, Sultanina (Thompson Seedless) and Malaga Grapes and Two Brands of Commercial Grape Juice. Esther Peterson Daniel and Hazel E. Munsell 59–70
Vitamin B—
antineuritic fraction, detection in peanut 853, 859
Complex and Its Components, Distribution in the Peanut. F. W. Sherwood and J. O. Halverson 849–860
content of grapes and grape juice, experiments 61–63, 69
determination in three varieties of spinach 769, 771
Vitamin C—
content of grapes and grape juice, experiments 63–66, 69
determination in three varieties of spinach 769–771
Vitamin G content of—
grapes and grape juice, determination 67–69
peanut, study 849–859
Vitamin Content, Spinach, Three Varieties. Hilda Black Kifer and Hazel E. Munsell 767–771
Vitamin, growth-promoting, in yeast, value 271
Vitamins, pellagra-preventing and antineuritic, relative quantities in peanuts 854–859
Vitis vinifera. *See* Grapes.

Page

Volume Tables, Tree, Construction, Simple Method. D. B. Demeritt and A. C. McIntyre 529–539

Water—
Bound—
in Plant Tissue, Methods of Determining. J. D. Sayre 669–688
measurement, methods 671–686
hydrogen-ion concentration, relation to presence of Anopheles larvae 394–396, 399
impounded, effect on Anopheles breeding 388
specific heat, table 680
surface, amount, influence on—
abundance of mosquito larvae 382–384
plankton content 385–386, 397
Weather, effect on growing period of barley 326–327
Wheat—
breeding for resistance to bunt 121–126
characters, coefficients of variability, relation to cytologic aberrations 822–825, 827–830
crossbreeding for rust resistance and desirable agronomic characters 797–830
heredity studies 797–830
inheritance studies 121–126
leaf rust. See *Puccinia triticina*.
microsporogenesis, strains, fragmentation 811–813
Marquillo—
germinal stability, cytologic study 797–830
origin and characters 797, 827–828
Marquis, germinal stability, cytologic study 797, 800–829

Page

Wheat—Continued.
Minnesota 2303—
microspores, occurrence of micronuclei 803–804, 829
origin and characters 797, 827–828
seedlings, inoculation with smut, experimental results 52–57
smut—
heterothallism and hybridization, studies 49–57
sporidia, formation of, description 51–53
strains—
cytologic aberrations, correlations between 820–822, 827–828, 829
Derived from Interspecific Crosses, Variability of, Cytologic and Genetic Studies. LeRoy Powers 797–831
Wheats, Resistance to Bunt, *Tilletia tritici*, Inheritance in Crosses of White Federation with Turkey. Fred N. Briggs 121–126

Yeast—
and Casein, Supplements to Corn and Soybean Rations, Effect on Rats and Swine. Charles L. Shrewsbury, Claude M. Vestal, and Sigfred M. Hauge 267–274
autoclaved, feeding to rats, experiments 854–859

Zaumeyer, W. J.: Comparative Pathological Histology of Three Bacterial Diseases of Bean 605–632
Zea mays. *See* Corn; Maize.